THE EFFECTS OF PROCESSING AND/OR ENZYME TO IMPROVE THE FEED VALUE OF WHEAT DISTILLERS DRIED GRAINS WITH SOLUBLES FOR TURKEYS

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ABSTRACT

Based on ongoing research, the poultry industry is utilizing increasingly more wheat distillers dried grains with solubles (wDDGS) as a feed ingredient. High fiber in wDDGS is a major factor contributing to reduced nutrient intake and digestion of nutrients in the diet. Hence, the research conducted looks at emerging technologies (e.g., enzymes and/or feed processes (extrusion) and/or wet feeding) to overcome the limitations in diets containing higher levels of wDDGS. The dietary treatments were evaluated by monitoring turkey performance and utilization of the nutrients. All dietary treatments in the respective experiments were formulated to meet or exceed the nutrient requirement of the Hybrid Converter turkey standards. With the exception of experiment 2 (0-72d) test diets were fed from 7-21d. In Experiment 1, 0 and 30% wDDGS diets were supplemented with protease (P+; 0.126 g/kg) or β-mannanase (M+; 0.05g/kg); further, diets containing 0, 10, 20 and 30% wDDGS with no enzyme were compared. A positive ($P<0.05$) main effect of 30% was reported for 21d body weight (BW) and feed conversion ratio (FCR). A significant main effect [21d apparent metabolizable energy (AME); 30%] and interactions [(enzymes x levels (0 and 30%); 21d AME and nitrogen retention (NR)] were found. A quadratic ($P<0.01$) response was found for FCR, that was superior for 30%. A quadratic ($P<0.01$) response was also found for both NR and AME; both were highest for 10% wDDGS diets. In Experiment 2, diets containing 0, 15 and 30% wDDGS with no enzyme were compared; further, the 30% wDDGS diet was supplemented with enzymes (protease or β-mannanase). Water intake per pen was monitored beginning at 7 d. There was no effect of dietary treatment on overall feed intake (FI) and body weight (BW). Total feed conversion ratio (FCR) ($P<0.05$; 0-72d) was significantly improved for birds fed 30% wDDGS regardless of enzyme treatment compared other dietary treatments. There were trends for higher water intake

(mL/b/d) with 30%P+ diets as compared to the other diets. The higher water intake may be a factor of the higher fiber in this diet, but it was exacerbated by only the protease enzyme. Experiment 3 evaluated the effect of extrusion (EX) and an enzyme cocktail (E; 0.5g/kg) on wDDGS. Diets containing 0, 15 and 30% wDDGS with/without enzyme were tested; further, the 15 and 30% wDDGS with/without EX and E were compared. There was no effect of EX or E on BW and FI. Feeding higher wDDGS (30%) depressed performance, but an improved NR and AME was recorded. In Experiment 4, a small study was conducted to evaluate if wet feeding (WF; 1.2 mL water: 1.0 g feed) of diets with 30% wDDGS would impact turkey poult performance. The WF significantly improved BW, FI and FCR. It is apparent from these studies that high levels of wDDGS were not detrimental to overall performance. The high levels of wDDGS with no loss of production would result in a higher demand of wDDGS for use in turkey diets. Overall, we saw no improvements in performance with individual supplementation of protease, β-mannanase or an enzyme cocktail. Neither was extrusion of wDDGS beneficial. Voluntary feed consumption is improved when diets are wetted before feeding.

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DEDICATION

This thesis is dedicated to my Mother (Theresah Fremah Gyan), a single mum who struggled through a lot of difficulties in bringing me up. Even though she could not go high in her academic career, she worked so hard to see me through my academic career. Her prayers, love and care has helped me in persuing my dreams.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
AIA	Acid insoluble ash
AID	Apparent ileal digestibility
AME	Apparent metabolizable energy
AMEn	Apparent metabolizable energy (nitrogen corrected)
AOAC	Association of Official Analytical Chemists
ASAE	American Society of Agriculture Engineer
BW	Body weight
CP	Crude protein
d	Day
DDGS	Distillers dried grains with solubles
D_{gw}	Geometric mean diameter
E	Enzyme cocktail
EX	Extrusion
EX-	Non-extruded
EX+	Extruded
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
FI	Feed intake
GE	Gross energy
HGCA	Home-Grown Cereals Authority
h	Hour
IDE	Ileal digestible energy

kcal/kg	Kilocalories per kilogram
L	Litres
M+	β-mannanase
ME	Metabolizable energy
MEn	Metabolizable energy (nitrogen corrected)
MMT	Million metric tonnes
N	Nitrogen
NDF	Neutral detergent fiber
NR	Nitrogen retention
NRC	National Research Council
NS	Non significant
NSP	Non-starch polysaccharide
N m	Newton metre
P	Phosphorus
P+	Protease
PROC GLM	SAS (1996) General Linear Models Procedure
SEM	Standard error of means
S_{gw}	Standard deviation of particle diameter
TMEn	True metabolizable energy (nitrogen corrected)
wDDGS	Wheat distillers dried grains with solubles
WF	Wet feeding

1.0 INTRODUCTION

The reliance on cereal grains for ethanol production is increasing due to factors such as increasing energy prices, uncertainties of petroleum supplies and the negative impact of fossil fuel on the environment. This challenges the animal feed industry in terms of supply and increase cost of cereals. Nonetheless, animals are capable of converting products not directly consumed by humans into high quality food. There is therefore the potential of utilizing the co-product from ethanol production (distillers dried grains with solubles) in the poultry industry (Olukosi et al., 2010).

The co-product DDGS from ethanol production is considered a good source of protein, but has limited energy due to fermentation of the starch of the base cereal used. Western Canada utilizes wheat as a major raw material for ethanol production. Zijlstra et al. (2010) highlights that feed processing and/or enzyme supplementation can be used to enhance the feeding value of low quality ingredients. However, basic knowledge about the nutritional value of wheat DDGS with enzymes and/or processing is limited.

Enzyme supplementation can increase nutrient digestibility and voluntary feed intake and reduce the risk associated with feeding these co-products to poultry (Zijlstra et al., 2010). Selecting enzymes in their right proportions and combinations to ensure effective utilization of wheat DDGS in diets of turkeys are concerns that need to be addressed. Most commercial diets for poultry are pelleted; however, knowledge about feeding extruded wheat DDGS to poultry species such as turkey is minimal. Additionally it is crucial to understand the interaction between these exogenous enzymes and processing (i.e., extrusion) on wheat DDGS.

A common practice in the swine industry that has not been well recognized in the poultry industry is wet feeding. Wet feeding according to Scott (2002) could reduce the limitation of feed ingredients resulting in increased voluntary feed intake. If enzymes, processing and/or wet

feeding can improve the nutrient value of wheat DDGS by reducing antinutritional factors, improve nutrient availability and consistency; then feeding value is increased whilst undigested and excreted nutrients are minimized.

The hypothesis of the current research was that higher levels of wheat DDGS can be included in turkey hen diets without detrimental effects on performance. Additionally, enzymes, extrusion and/or wet feeding can reduce the limitations of feeding higher levels of wheat DDGS by adding value (i.e., improving nutrient digestibility and availability) to the co-product. To address these issues, experiments were designed to test the nutrient digestibility and inclusion levels of wheat DDGS; and to investigate if enzyme, extrusion and/or wet feeding can add value to wheat DDGS utilization which will further increase the interest in using this co-product in poultry production.

2.0 LITERATURE REVIEW

2.1. Feeding co-products to poultry

Feed accounts for the greatest cost for animal production and is determined by costs of ingredients and the capacity to meet nutrient requirements (Leeson and Summers, 2001; Yegani and Korver, 2008; Leeson, 2012; Kerr and Shurson, 2013). Using DDGS as a feed ingredient for animals has been practiced for years. Beneficial effects of using DDGS include less reliance on cereal grains that have been redirected to ethanol production. Using DDGS as an alternative ingredient for livestock could also be a strategy to offset the higher cost of production. Use of these co-products as a feed stock for livestock will play a significant role in ensuring the sustainability of the ethanol industry; as the sales of this ingredient (DDGS) contributes between 10-20% to the industries total income (Rosentrater, 2012).

2.2. An overview of bioethanol production

Expansion in the biofuels industry has been primarily driven by the need for a supplemental energy (Smith et al., 2006; Bruce et al., 2007; Świątkiewicz et al., 2013). Thus biofuel has a comparative advantage over petroleum gas in terms of renewability; cleaner burning, more energy and lower CO_2 emission (Lumpkins et al., 2004 Swiatkiewicz and Koreleski, 2008). The typical feedstocks used for ethanol production contain high levels of fermentable sugars and include corn, wheat, and cane or beet sugar. Figure 2.1 provides information of the starch content and the corresponding ethanol yield of some cereal grains. The starch and the ethanol yields are quite comparable according to this figure.

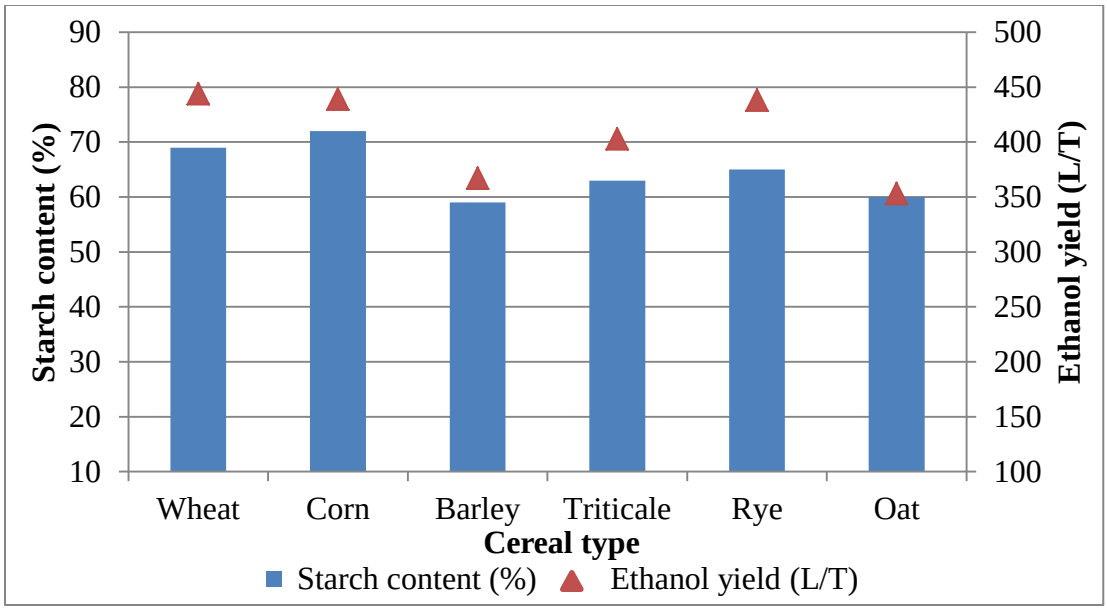

Figure 2.1: Starch content (%) of cereal type and their corresponding ethanol yield (L/T) Adapted from Smith et al. (2006)

Corn is the primary cereal used for ethanol production in the United States, whereas wheat is most commonly used in Western Canada and Europe (Bruce et al., 2007; Cozannet, 2009; Avelara et al., 2010). Ethanol production from wheat accounts for ~1.4 MMT of wheat distillers dried grains with solubles (Ethanol Producer Magazine, 2013). The outlook for Canadian ethanol production is illustrated in Figure 2.2. According to this figure, there has been a trend for increasing production of ethanol with greater than a two fold increase between 2007 and 2011. In recent years (2011-2012), there was a slight reduction in ethanol production in Canada.

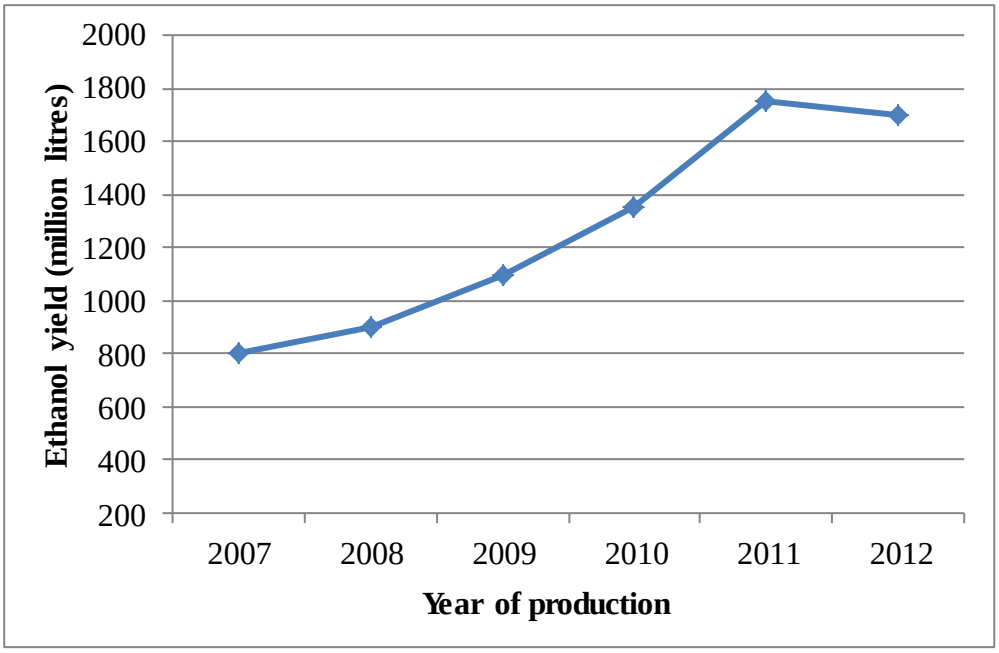

Figure 2.2. Canadian Ethanol Production Outlook
Adapted from Renewable fuel association (http://ethanolrfa.org/pages/World-Fuel-Ethanol-Production) Assessed on November, 21st 2013

2.2.1. Process of bioethanol production

The process of ethanol production involves a series of steps including milling, gelatinization and liquefaction, saccharification, fermentation, distillation and dehydration, and stillage separation. The process of bioethanol production as described by Smith et al. (2006) is summarized below.

1) Milling: The grain used in ethanol production is first ground (e.g., dry or wet) to reduce particle size and increase surface area. With dry grinding, the whole grain is ground without separation of its components. Whereas, with wet grinding, the grain is separated into its components: starch, fiber, protein and germ. Even though the dry grind process is cheaper, the wet grind results in increased processing efficiency due to the higher proportion of starch fermented and reduced requirement for drying the DDGS.

2) Gelatinization and liquefaction: This involves the application of water and heat to facilitate gelatinization and enhance accessibility by enzymes and reduce unwanted microbial growth. Starch gelatinization is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat. The milled product is mixed with water and heated at a temperature between 120 to 150°C for about 15 to 20 minutes.
3) Saccharification: An α-amylase enzyme is then added after cooling to about 90 to 100°C. The enzyme hydrolyzes the starch into smaller sugars and also reduces the viscosity of the mash produced. After further cooling between 80 to 90°C, amyloglucosidase is added. Amyloglucosidase removes successive glucose residues at the ends of the starch molecules.
4) Fermentation: After further cooling, yeast is added to ferment sugars into ethanol and carbon dioxide. The process of fermentation takes approximately 2-3 days at a temperature ranging between 30-35°C.
5) Distillation and dehydration: This process results in the separation of the ethanol from water and other contaminants in the mash. Molecular sieves that absorb only water are used, so as to produce quality ethanol at the end.
6) Stillage separation: After distillation the remaining water is reduced by centrifugation to produce solids and liquids (stillage). The stillage is dried to obtain syrup and usually combined with wet grain fraction and the DDGS is dried further to below 12-15% moisture to minimize bacterial growth.

A diagrammatic representation of this process is shown in Figure 2.3.

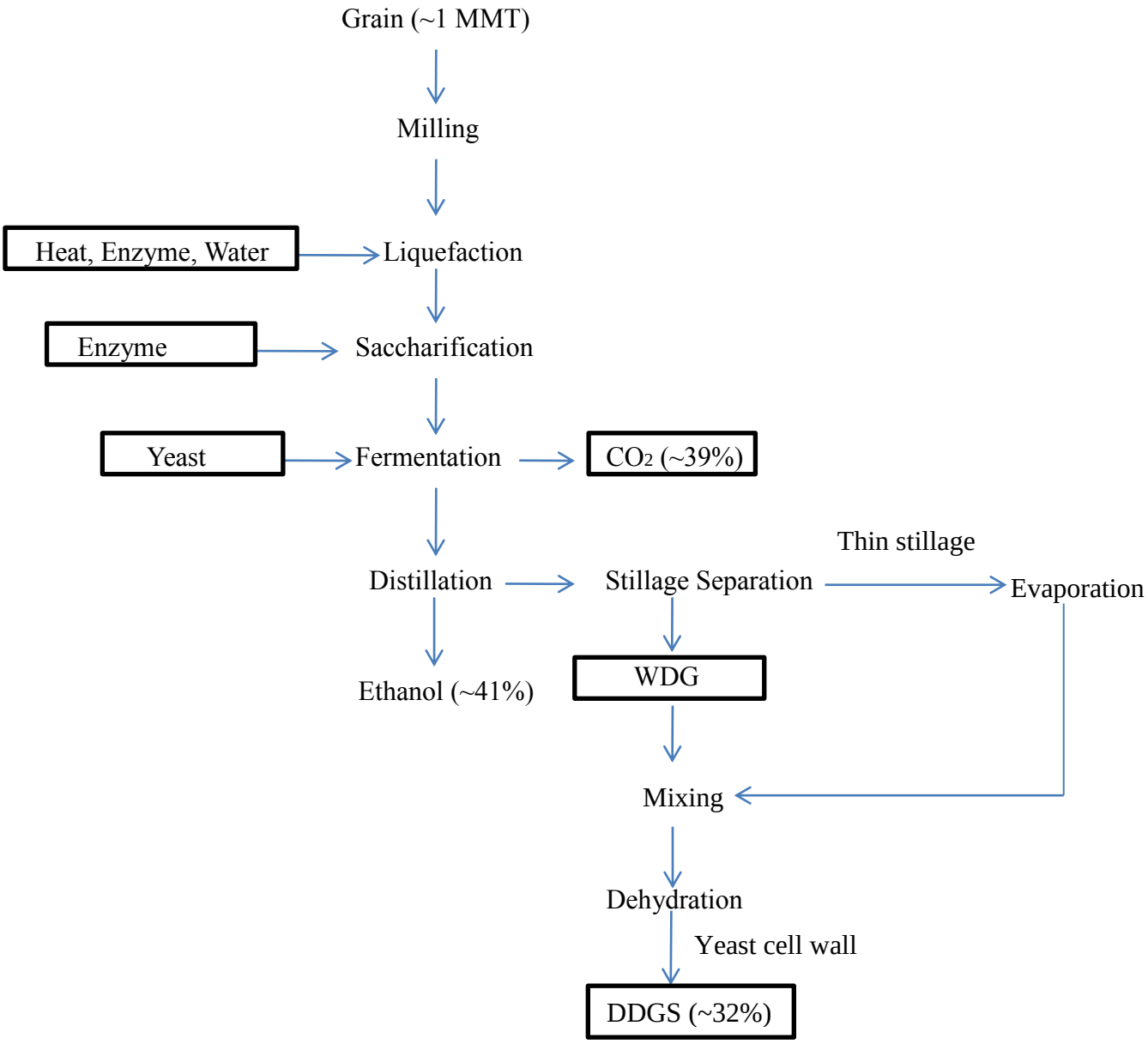

Figure 2.3: An overview of fuel ethanol production (adapted and modified from Smith et al., 2006)

2.2.2 Effects of bioethanol processing on nutritional composition of the co-product (DDGS)

As the bioethanol industry continues to evolve, new manufacturing processes will undoubtedly change how ethanol is produced (Lumpkins et al., 2004; Bruce et al., 2007; Oryschak et al., 2010b; Rosentrater and Liu, 2012). Several variables inherent in the production process such as types of grain, different processes for fermentation, enzymes used, drying

temperature and duration can substantially impact the nutritional value of DDGS (Spiehs et al., 2002; Fastinger et al., 2006; Bruce et al., 2007; Martinez-Amezcua, 2007; Pahm et al., 2008; Abdel-Raheem et al., 2011; Bolarinwa and Adeola, 2012; Rosentrater and Liu, 2012).

Starch is degraded to release sugar which is then fermented by yeast to produce ethanol and carbon dioxide (Bruce et al., 2007). Some residual starch (~1-3%; wheat DDGS; Bruce et al., 2007) remain in the co-product. Overall, the ethanol production process results in an increased concentration (~3x) of the other unfermented nutrients in the co-product compared to the major cereal (Bruce et al., 2007; Rosentrater and Liu, 2012). Enzymes are increasingly being used in ethanol production to enhance fermentation (Smith et al., 2006 and Bruce et al., 2007). For instance, the use of hemicellulase enzyme is intended to reduce the non-starch polysaccharide (NSP) levels and energy cost of processing while increasing the quantity of ethanol produced (Bruce et al., 2007). Wheat is generally known to contain higher levels of NSPs (e. g., pentosans; ~5-8%; Choct and Kocher, 2000). With enzymes to hydrolyze NSP, the final DDGS will have less soluble NSP and reduced problems with digesta viscosity (Carre and Brillouet, 1986; Choct and Kocher, 2000). Protease enzymes are also used in ethanol production to breakdown proteins to peptides and amino acids and further improve fermentation (Bruce et al., 2007; Hruby, 2012). According to Hruby (2012) protease added to the soaked grain during the wet milling process increases starch recovery and total ethanol output. This reduces the energy content of the co-product. Another major concern contributing to the energy reduction on DDGS is the fat extraction process (with corn); commonly practiced in modern ethanol production (Wisner et al., 2013).

According to Bruce et al. (2007) the degradation of starch results in the release of more pentose sugars, which could lead to the formation of Maillard reaction (i.e., irreversible binding

of free sugars with amino acids, in particular lysine) through the heat generated during drying (Fastinger et al., 2006; Bruce et al., 2007).

The yeast used during the process of fermentation contributes to the total protein content of DDGS (Ingledew, 1995). According to Belyea et al. (2004) a greater proportion of the amino acid in DDGS is of yeast origin. The yeast biomass is also a significant source of mannan (~6%) which would potentially be an antinutritional factor (Radfar et al., 2013).

Even though antibiotics are used in the bioethanol industry, little is known about antibiotic residues in DDGS. According to Compart et al. (2013) major antibiotics present in DDGS are penicillin G, virginiamycin M1, tylosin, erythromycin, and tetracycline. Due to the susceptibility of fermentation via yeast to bacterial infection, these antibiotics are used as a means of preventing competition between yeast and bacteria for nutrients (Compart et al., 2013). The competition could result in decreased fermentative capacity of the yeast thereby decreasing ethanol yield.

Antibiotic residues in distillers grains could potentially serve as preventive measures against pathogens to the livestock consuming those co-products (Compart et al., 2013). Compart et al. (2013) examined the concentrations of antibiotic residues and its biological active level in distillers dried grains (DDG) and distillers wet grains (DWG). They reported the concentration of erythromycin (0.35 mg/kg) and penicillin G (0.11 mg/kg) for DDG and a concentration of erythromycin (0.35 mg/kg) and tetracycline (0.11 mg/kg) for DWG. According to these authors, these concentrations are below the minimum requirements (10.2 mg/kg; erythromycin and 55.1 mg/kg; penicillin G) in turkey diets. The results indicated that 13% of all samples contained low ($\leq$1.12 mg/kg) antibiotic concentrations. Compart et al. (2013) reported that only one sample extract did prevent the growth of *Escherichia coli* at 104 CFU/mL, but this sample had no

detectable concentrations of antibiotic residues. According to Jacob et al. (2008) the temperatures used in the distillation process are adequate to reduce the antimicrobial residue in the co-product.

2.3 Nutrient content of DDGS

Table 2.1 shows the chemical composition of wheat and their respective co-products (DDGS). With the exception of the starch content (Cozannet et al., 2009), all other chemical constituents increases in the co-products (wheat DDGS) compared to wheat. Distillers dried grains with solubles as a feed ingredient can differ between feedstock and within any given processing facility. Nonetheless, the protein content and to limited extent energy, makes DDGS an attractive ingredient in animal feed (Belyea et al., 2010). Thacker and Widyaratne (2007) reported a crude protein (CP) content of wheat DDGS to be 35.7%, similar to the reported crude protein in Table 2.1. Ergul et al. (2003) found the CP digestibility of wheat DDGS to range from 76-85%.

Sulfuric acid (H_2SO_4) is supplied at different phases in the process to regulate the pH for the carbohydrases and the yeast at various optimum levels (Rosentrater and Liu, 2012). This increases the sulfur (S) level in the co-product. Additionally, sulfur in the DDGS is provided by the yeast and well water used in the production of ethanol (Rosentrater and Liu, 2012). However, not much is done to quantify the concentration of sulfur in DDGS.

Table 2.1: Chemical Composition of Wheat DDGS; Comparison with Wheat

	Nyachoti et al. (2005)		Cozannet et al. (2009)		Oryschak et al. (2010b)		Bolarinwa and Adeola. (2012)	
Item	Wheat	wDDGS	Wheat	wDDGS	Wheat	wDDGS	Wheat	wDDGS
Dry matter	92.35	95.64	86.8	92.7	87.40	91.66	89.9	93.90
Gross energy (kcal/kg)	4036	4896	3870	4460	5086	5160	4006	4181
Crude protein	13.31	40.37	12.10	36.60	17.15	39.20	9.50	37.13
Ash	1.62	4.43	1.20	5.00	2.20	5.50	-	-
Crude fat	1.50	3.68	1.70	4.40	1.69	7.04	1.66	6.00
Crude fiber	-	-	2.50	7.60	2.43	7.82	-	-
NDF	11.81	30.65	14.30	30.10	15.02	46.81	10.85	27.50
ADF	4.82	13.15	3.60	10.70	2.97	10.48	2.62	11.40
Starch	-	-	69.70	5.10	-	0.00	-	-
Sugar	-	-	2.80	4.00	-	-	-	-
Calcium	0.06	0.16	-	-	0.09	0.24	0.05	0.18
Phosphorus	0.37	0.85	-	-	0.44	0.99	0.29	0.85

2.3.1 Proteins and Amino acids

Table 2.2 summarizes the crude protein and essential amino acid (aa) content of wheat and wheat DDGS. According to the table, the aa profile of the wheat and their corresponding co-product (DDGS) are quite comparable. However, lysine and arginine are lower for DDGS compared to the respective cereal source. Additionally, there is a larger variation between lysine (1.7 - 3.0%) and arginine (3.7 - 4.6%) than for other aa.

Table 2.2: Concentration of crude protein (CP) and essential amino acid (aa; % N*6.25) in wheat, wheat DDGS

		Wheat DDGS	
	Wheat	Mean	Min - Max
CP, % DM	12.1	36.6	32.7-39.2
Essential AA			
Arginine	5.1	4.3	3.7-4.6
Histidine	2.3	2.1	1.9-2.2
Lysine	2.9	2.3	1.7-3.0
Phenylalanine	4.7	4.5	4.3-4.6
Leucine	6.8	6.5	6.2-6.8
Isoleucine	3.6	3.5	3.4-3.5
Valine	4.4	4.3	4.2-4.4
Methionine	1.6	1.5	1.4-1.5
Threonine	3.1	3.0	2.9-3.1
Tryptophan	1.2	1.1	1.0-1.2
Total	35.7	33	31.2-34.4
Non-essential AA	61.9	56.3	53.9-57.7

Adapted from Cozzanet et al. (2009)

Knowledge of the amino acid content of a feedstuff is very important to the animal industry (Ravindran et al., 1999). Lysine and arginine are very critical when diets are formulated with wheat DDGS (Cozzanet et al., 2009). Cozzanet et al. (2009) has indicated that there is a higher variability in wheat DDGS protein levels compared to corn DDGS. However, the average protein level is higher for wheat than corn DDGS. Lysine is easily damaged or made indigestible when materials are heated as would be the case for wDDGS (Bruce et al., 2007;

Swiatkiewicz et al., 2013). As indicated earlier, this is due to the formation of complexes between the sugars and the amino acids (primarily lysine) reducing its digestibility (Bruce et al., 2007; Batal and Bregendahl, 2012). Lumpkin et al. (2004) showed that increasing the levels of corn DDGS results in marginal lysine deficiency, which was more pronounced in younger birds due to their higher demand for lysine. Fastinger et al. (2006) showed that light colored DDGS had higher lysine content with higher lysine digestibility in broilers. Similarly, Cromwell et al (1993) reported an increase in lysine (in nine sources of DDGS) digestibility (86%) when pigs were fed light-colored corn DDGS as compared to dark colored corn DDGS (62%). A report by Noblet et al. (2012) has indicated the concentration of lysine nonheat-damaged corn DDGS to range between 3.1 and 3.3%, while heat-damaged corn DDGS is low at 2.10%. The available (reactive) lysine content was not measured in the experiments reported and thus effects of processing on available lysine in diets are not known.

2.3.2 Energy

The energy value for corn DDGS is 2480 kcal/kg of MEn and 3097 kcal/kg of TMEn (NRC, 1994). No value is available in the NRC on the ME of wheat DDGS. Ewing (1997) published an ME of wheat distillers dried grain for poultry of 2651 kcal/kg. Thacker and Widyaratne (2007) also reported the ME of wheat DDGS to be 2387 kcal/kg in broiler diets. The study by Oryschak et al. (2010) showed a higher apparent ileal digestibility of gross energy for corn DDGS as compared to wheat DDGS at 30% inclusion, even though the CP and amino acids were higher in wheat DDGS as compared to corn DDGS. An evaluation of the TMEn of corn DDGS samples by Fastinger et al. (2006) using adult caecectomised roosters resulted in values ranging from 2484 to 3047 kcal/kg. In a similar experiment conducted by Batal and Dale (2006), a TMEn values of 2490 to 3190 kcal/kg were found for corn DDGS. Adeola and Zhai (2012)

reported a linear decrease in digestibility of dry matter and ileal digestible energy when corn DDGS was substituted for corn at higher levels (30 to 60% of diet). The apparent metabolizable energy (AME, kcal/kg DM basis) and AMEn (corrected for nitrogen; kcal/kg DM basis) of wheat DDGS is shown in the Table 2.3. The AME and the AMEn for Vilarino et al. (2007) are higher compared to other reported values in the table. These AME values by Vilarino et al. (2007) were estimated using pelleted diets (i. e., may have increased energy digestibility) compared to the other studies in which mash diets were used.

Table 2.3: Apparent metabolizable energy (AME kcal/kg) and apparent metabolizable energy corrected for endogenous nitrogen excretion (AMEn kcal/kg) of wheat DDGS

	AME (kcal/kg)	AMEn (kcal/kg)
Roosters		
Vilarino et al (2007)	2701	2672
Meteyer et al (2009)	-	2345
Cozzanet et al (2010)	2464	2469
Broilers		
Meteyer et al (2009)	-	2047
Cozzanet et al (2010)	2421	2371
Layers		
Cozzanet et al (2010)	2412	2300
Turkey		
Cozzanet et al (2010)	2314	2164

Adapted from: Newkirk (2011) (Wheat DDGS feed guide, 1st edition)

2.3.3. Minerals and vitamins

Distillers dried grains with solubles has approximately 0.9% phosphorus (P) (Spiehs et al., 2002) with a bioavailability of 54-100% for poultry (Martinez-Amezcua et al., 2004; Lumpkins and Batal, 2005). The yeast used for fermentation produces small quantities of phytase which aids in increasing the bioavailability of the P (Martinez-Amezcua et al., 2004). Martinez-Amezuca et al. (2004) reported an average of 0.73% P content from 20 corn DDGS samples. According to Martinez-Amezuca et al. (2004) the heat generated during the drying

process could have a positive effect on P availability. This indicates that both fermentation and heating has a significant role in improving P availability.

Concentration of sulfur (S) in DDGS is higher than in the respective fermentation source (Rosentrater, 2012) due to the use of H_2SO_4 to manipulate pH to improve fermentation. Higher concentration of S in DDGS diets will increase excretion, but is also associated with reduced thiamine availability (Rosentrater, 2012). Additionally higher S can reduce the absorption of calcium and some trace minerals that will negatively affect skeletal and egg shell strength (Leeson and Summers, 2001).

Most scientific publications have been focused on energy, proteins, and to a limited extent mineral content of DDGS; however, there is inadequate information on the estimated vitamin levels in DDGS (Jung et al., 2013). Fermentation of ingredients does not only result in increasing the cconcentration of protein and amino acids, but also vitamins (Ochanda et al., 2010). Orchanda et al. (2010) reported a significant increase in the concentration of B-vitamins by fermentation of sorghum using a natural lactic acid method. Jung et al. (2013) reported an average value of vitamin E (α-tocopherol) to be 6.8 mg/kg in 6 corn DDGS samples. Additionally, the average thiamine and riboflavin values were 7.7 and 2.3 mg/kg, respectively. Average concentrations of pyridoxine (3.5 mg/kg) and pantothenic acid (10.9 mg/kg) were also reported.

2.4. Use of DDGS in monogastric animal production

The increased information published on the utilization of DDGS for poultry ((Noll et al., 2001; Lee et al., 2003; Świątkiewicz and Koreleski, 2007; Thacker and Widyaratne., 2007; Bregendahl, 2008; Cozannet et al., 2009; Lim et al., 2009; Avelar et al., 2010; Chevanan et al., 2010; Fallahi et al., 2010; Oryschak et al., 2010a; Dozier, 2012; Leeson et al., 2012) enables better formulation accuracy by industry. Until recently, lower levels were recommended in

poultry diets (Noll et al., 2001; Waldroup et al., 2007; Martinez-Amezcua et al., 2007; Swiątkiewicz and Koreleski, 2007; Leytem et al., 2008; Olokosi et al., 2010) due to limitations such as high fiber (Cozzanet et al., 2010; Abdel-Raheem et al., 2011).

Thacker and Widyaratne (2007) substituted wheat and soybean meal with wDDGS at 5, 10, 15 and 20%. They reported that the highest level (20%) of wDDGS resulted in an increase in mortality. These authors recommended a maximum inclusion in broiler diet at 15%. Consistent with this, Oryschak et al. (2010a, b) reported that wheat and triticale DDGS-based diets were best limited to 10% without significant loss in performance. Lumpkins et al. (2004) fed diets containing corn DDGS at 0, 6, 12 and 18% to broiler and reported no significance difference in feed intake between dietary treatments. However, feed efficiency (gain:feed) was lower for the highest level of DDGS (18%) in the starter phase but not different for grower and finisher phases. They speculated that, the decrease performance in the starter phase was due to the overestimation of lysine content in the DDGS which resulted in an error in feed formulation. If energy level is kept constant, up to 25% corn DDGS could be included in boiler diet (Waldroup et al., 1981). Wang et al. (2007) investigated the effects of corn DDGS (5, 10, 15, 20, and 25%) on broiler performance; and diets were formulated on digestible amino acid bases. They reported that inclusion of DDGS up to 25% does not have adverse effect on growth rate but diets containing 25% DDGS recorded higher FCR.

Cozannet et al. (2010) reported a decrease in average daily gain by incorporating wheat DDGS at 25% in turkey diets; feed intake was however not affected. This disagrees with Vilarino et al. (2007) who reported that 20% wheat DDGS reduced feed intake and body weight. Additionally, Roberson (2003) investigated the impact of corn DDGS on turkey hens and reported no depression on performance at an inclusion rate of 10%. Similarly, body weight and

feed conversion ratio were improved when turkeys were fed diets containing 20% corn DDGS (Noll et al., 2004; Noll and Brannon, 2006).

Alenier and Combs (1981) showed a higher performance for diets containing 10% or 15% DDGS over a corn-soy diet in layer hens. Proper balancing for lysine and energy level would be a contributory factor to a successful incorporation of 25% DDGS in diet formulation without negative effects on bird's performance (Parsons and Baker, 1983). Parson et al. (1983) recorded a replacement of 40% soybean protein with DDGS when sufficient amount of lysine was provided in the diet. Furthermore, an inclusion level of 20% wheat or barley DDGS did not show any negative impact on performance of laying hens (Nisi, 1990). Waldroup et al. (1981) indicated that substitution of 25% DDGS for corn and soybean meal depressed performance due to reduce lysine digestibility.

2.5 Limitations for use of DDGS in poultry diets

2.5.1 High fiber

The major constituent of dietary fiber in poultry diets is NSP; comprising of cellulose, hemicellulose, pectin, fructans, glucomannans, galactomannans, mucilages (Slominski 2011; Kerr and Shurson, 2013). High fiber content of DDGS undoubtedly limits its inclusion in poultry diet (Spiehs et al., 2002; Lee et al., 2003; Lumpkins et al., 2004; Lim et al 2009; Fallahi et al., 2013). Although high fiber in diets of poultry can have negative effects on voluntary feed intake and digestibility, it was traditionally used as a diluent in diets for animals (Mateos et al., 2012). Addition of fiber to diets enhances the development of the digestive organs (i.e., gizzard) and reduce passage rate of digesta thereby increasing nutrient susceptibility to enzymes (Mateos et al., 2012). Under practical conditions, effective utilization of fiber is dependent on physical and chemical characteristics of the fiber type, source of fiber, and the ingredient making up the diet (Lee et al., 2003; Mateos et al., 2012). Duke (1996) reported an increase in nutrient

digestibility when turkeys were offered a high fiber diet. This suggests that, turkeys have the capacity to tolerate incorporation of DDGS at higher levels in their diet. This may be an indication of higher fiber digestion in the caeca. The caeca in poultry provides a longer resident time and more fermentation capacity. Therefore, the caeca is generally known for degrading materials (e. g., fiber) that escapes digestion in the lower digestive tract (Remington, 1989; Klasing, 1998; Lee et al., 2003; Svihus et al., 2013). The fermentation of fibrous feedstuffs in the caeca enhances the production of short chain fatty acids (Kerr and Shurson, 2013; Svihus et al., 2013) contributing to increased energy digestibility.

2.5.1.1 Effects of feeding high fiber diets on intestinal tract

The morphological and physiological development of the intestinal tract is associated with early access of poultry to feed (Potturi et al., 2005). A number of authors (Hetland and Svihus, 2001; Gonzalez-Alvarado et al., 2007; Jimenez-Moreno et al., 2009; Mateos et al., 2013) have reported that significant increases in intestinal size are associated with higher fiber diets. These diets could either positively (Lee et al., 2003) or negatively (Yegani and Korver, 2008) influence gut development. Increase in the length and weight proportionally causes a higher demand for energy and amino acid for maintenance of the gut (Baldwin et al., 1980; Wenk, 2001). Consequently, methods to improve fiber digestion would reduce these negative effects of fiber on animal metabolism **(**Kerr and Shurson, 2013). Barekatain et al. (2013) fed sorghum DDGS in a ratio of 15 and 30% and recorded an increase in relative weights of proventriculus, gizzard and small intestine (duodenum, jejunum and ileal). By feeding a diet containing soybean hulls (3% inclusion) to chicks, Gonzalez-Alvarado et al. (2007) found a heavier gizzard and ceca and a shorter small intestine. Similarly, Hetland and Svihus (2001) observed an increased gizzard weight when broilers were offered diets containing 10% oat hulls. The increase in

mechanical stimulation, influenced by active grinding (due to higher levels of fiber in DDGS) might possibly be the reason for this effect (Owusu-Asiedu et al., 2006; Barekatain et al., 2013). One strategy to reduce the negative impact of diets on gut measurement is wet feeding. This has been demonstrated by Afsharmanesh et al. (2006) who reported significant increases in performance with a reduction in relative gizzard size and lower intestinal tract measurement when broilers were offered a wheat-based diet (either Durum or Hard Red Spring) in wet form.

The soluble fractions of fiber are associated with more viscous digesta that results in reduced absorption of nutrients, bacterial overgrowth in the upper digestive tract and associated with increases in wet litter. However, as indicated earlier these sources of fiber are reduced through the fermentation process. Gonzalez-Alvarado et al. (2007) postulated that diets containing higher fiber contents have a longer residence time in the gut and hence an expected increased digestion. However severity of the effect is dependent on fiber source (Gonzalez-Alvarado et al., 2007) and section of the gut under consideration (Kerr and Shurson, 2013).

2.6 Effects of dietary ingredients inclusion on water consumption

Water consumption is not routinely monitored or reported in animal trials (Viana et al., 2010), but is a critical nutrient with intake usually twice that of feed. Dietary ingredients and diet formulations contribute significantly to water intake and drinking pattern of animals (Shaw et al., 2006). Hence the requirements of water for animals may differ (Schlink et al., 2010) depending on diet offered (Patience et al., 2005, Shaw et al., 2006; Schlink et al., 2010). There is greater requirement of water for metabolism of protein compared to carbohydrates and fats. It is critical to recognize that excess water consumption could result in wet litter and predispose birds' to disease challenges and increase production cost. Shaw et al. (2006) reported an increase in the amount of urine excreted by pigs fed a high protein diet as a means of excreting excessive nitrogen produced during metabolism of excess protein for energy. Wheat DDGS

contains approximately 35.7-40.0% CP (Bruce et al., 2007; Thacker and Widyaratne, 2007). Inaccurate feed formulation resulting from variability in DDGS nutritional composition could lead to over estimation of nutrient (e.g., protein) content. Therefore animals might tend to increase their water intake to enhance protein metabolism. There have also been associations with increased water uptake when diets contain high levels of soluble NSP (Daskiran et al., 2003).

2.7 Improving the feed value of DDGS

The limitations of feeding co-product (e.g., DDGS) have intensified the interest of feed processing methods and exogenous enzymes to improve feeding value. Some of these technologies include the use of enzymes; predominantly used in poultry and swine production. Additionally, although much is known about extruding of human, aquaculture and pet foods there is little information on extruding commercial poultry diets or the impact of this technology on individual ingredients, such as DDGS. Others have also showed that wet feeding was useful in overcoming limitations in voluntary feed intake and improving poultry performance. However, wet feeding has not been adopted by the poultry industry as it has by pig producers.

2.7.1. The use of exogenous enzymes

Monogastrics are incapable of producing sufficient amounts of some enzymes to reduce antinutritive effects and increase nutrient digestion of some feed ingredients. Exogenous enzymes can degrade complex carbohydrates and increase the utilization of feed ingredients (Cowieson et al., 2006; Kalmendal and Tauson, 2012).

2.7.1.1 Enzyme activity levels, substrate availability and enzyme source

The potential efficacy and the consistency of enzyme activity are proportionally related to substrate availability (Zijlstra et al., 2010). According to Choct (2006) "substrate specificity depends largely on the source of the enzyme". Exogenous enzymes are produced either from

bacterial or fungal sources through fermentation (Adeola and Cowieson, 2011). Studies using enzymes have shown variability in results with respect to the fermentation source. A keratinase enzyme originally purified from the growth medium of *Bacillus licheniformis* was fed in a corn/soy-based broiler starter diet and found improved performance (Odetallah et al., 2003); although there were not consistent responses in three separate studies reported. This might be associated to the increase in amino acid or CP concentrations in their diets (Odetallah et al., 2003; Adeola and Cowieson, 2011). Keratinase (broad-spectrum protease enzyme) degrade proteins into simpler polypeptide components, which increases their accessibility by digestive enzymes (Odetallah et al., 2003). On the other hand Simbaya et al. (1996) used a protease purified from *Streptomyces griseus* and saw no significant effects on performance. Ghazi et al. (2003) also fed protease enzyme from either *Aspergillus niger* or *Bacillus subtilis* and indicated a better utilization of nutrients. However, protease fermented from *Bacillus subtilis* showed a decreased TME as compared to those from *Aspergillus niger*.

2.7.1.2 Efficacy of exogenous protease

Generally, proteases hydrolyze bonds within the complex protein structure (Barletta, 2012; Isaksen et al., 2012) and produce peptides and/or amino acids. Exogenous protease is seldom fed in isolation, but rather in combination with other carbohydrase enzymes (Cowieson and Adeola, 2005; Cowieson and Ravindran, 2008; Olukosi et al., 2010); hence little is known about specific protease supplementation (Isaksen et al., 2012; Barekatain et al., 2013) to co-products such as DDGS. Ghazi et al. (2002) showed improved energy and nitrogen digestibility in broilers fed a soybean based diet supplemented with protease. Similar results were reported by Ghazi et al. (2003) as indicated in the previous section. According to Ghazi et al. (2003) the protease was fed with an α-galactosidase also containing pectinase, xylanase, cellulase, and

amylase activity; the latter activities may have contributed to the improved performance. Kalmendal and Tauson (2012) fed broilers a corn-soybean diet supplemented with protease and found improved FCR and AMEn. However, body weight and feed intake were negatively affected with protease supplementation. The above studies indicate that inherent characteristics of the different proteases might be responsible for producing contradictory responses. Additionally, Odetallah et al. (2005) saw a beneficial effect of protease in a corn/soy-based diet up to 22 d of age in broiler diet, but these positive effect disappeared at market age (43d of age). According to Odetallah et al. (2005), the protease might have caused an increasing the availability of the amino acids present in the diet, thus suggesting a protein-sparing effect by the protease enzyme. Barekatain et al. (2013) reported a significant improvement in broiler performance (bodyweight and feed intake) when fed diets containing sorghum DDGS supplemented with protease. Barekatain et al. (2013) also reported a significant increase in amino acid digestibility (His, Glu, Pro and Met) with protease supplemented to sorghum DDGS.

2.7.1.3 Efficacy of exogenous β-mannanase

Glucomannan, galactomannan, glucogalactomannan and glucurono-mannan are naturally occurring mannans in non-starch polysaccharides (Jackson, 2012). Jackson (2012) describes mannan and heteromannan as basic components of the hemicellulose portion of plants. Mannan is an anti-nutritional effect found in soybean meal (Tucker et al., 2004). As indicated earlier, the yeast used in fermentation of DDGS contains ~6% mannan that could potentially contribute to antinutritional effect of DDGS (Tucker et al., 2004; Radfar et al., 2013). The carbohydrase enzyme β-mannanase is reported to improve performance of animals fed soybean-based diets (Odetallah et al., 2002; Pettey et al., 2002; Jackson et al., 2008; Mehri et al., 2010). Kong et al. (2011) supplemented a corn-soybean based diet with β-mannanase and reported an improved

total tract digestibility of energy and AME in broilers. The improvement in nutrient digestibility however, did not result in a positive impact on performance. The reason for the discrepancies between nutrient availability and the lack of a positive change in performance are not known. McNaughton et al. (1998) reported a significant increase in average daily gain, and feed efficiency of broilers fed a soy-based diet supplemented with β-mannanase. In comparison, Ouhida et al. (2002) also reported a poor performance in terms of feed intake, body weight or feed conversion ratio when broilers were fed a soybean diet containing β-mannanase.

2.7.2 Enzyme combinations

A multi-enzyme supplement or an enzyme cocktail has a wide range of activities and could effectively degrade the complex matrixes of fibrous carbohydrates or undigestible cell wall components of feed ingredients (Choct et al., 2004; Cowieson and Adeola, 2005; Tahir et al., 2008; Emiola et al., 2009; Adeola and Cowieson, 2011; Kalmendal and Tauson, 2012; Kerr and Shurshon, 2013). According to Meng and Slominski (2005) diet composition may be responsible for the positive effects of these enzymes. Improvement in nutrient digestibility in broilers and pigs offered a diet consisting of a combination of xylanase, amylase, protease or β-glucanase, xylanase and amylase has been reported (Inborr et al., 1993; Olukosi et al., 2010). Emiola et al. (2009) reported that a multi-enzyme complex that supplied xylanase, β-glucannase, and cellulase in pigs fed a diet containing wheat DDGS improved apparent ileal digestibility of nitrogen and gross energy and also increased performance. Cowieson and Adeola (2005) investigated the additive effects of protease with xylanase, amylase and phytase. These enzymes were added to a corn-soybean meal diet containing rye (serving as the negative control) and a positive control diet (corn-soybean meal; without any enzyme addition). An improvement in the digestibility of the negative control diet with enzymes was noted. However, supplementation of

enzymes to the negative control (rye-based diet) with was not effective in fully returning performance (FCR or BWG) compared to that of the positive control diet.

2.8 Feed extrusion effects on feed value

Extrusion technology is a process that permits the use of temperature, moisture, pressure, shear, and mixing with variable time to modify the physical and nutrient structure of diets and/or ingredients (Fallahi et al., 2013). This process is used extensively for human, aquaculture and pet foods (Chevanan et al., 2009; Hood-Niefer and Tyler, 2010; Ayadi et al., 2011; Fallahi et al., 2013; Muthukumarappan, 2012).

2.8.1 Extrusion of feed

Vukic-Vranjes et al. (1994) observed no improvement in performance by feeding extruded wheat and corn to broilers up to 21d. Whereas, Gracia et al. (2003) reported that steam cooking barley diets at 99±2°C for 50 minutes increased body weight at 8d; however, the benefit was not found at later ages. Additionally, Garcia et al. (2008) reported that feeding barley-based diets produced with an expander (120°C, pressure; 30 bars and moisture; 19.3% for 5 sec.) resulted in an improved body weight and feed intake until 21d; again, this was not sustained to the end of the trial. On the contrary, earlier studies by Vukic-Vranjes and Wenk (1995) showed that feeding broilers an extruded (i. e., between temperatures of 120-130°C, with a pressure of 80 bar and 23% moisture) barley-based diet does not positively influence the performance in any of the growth phases.

Information regarding the impact of extruded DDGS on intestinal tract measurements is scarce. Gonzalez-Alvarado et al. (2007) found that steam-cooking of corn (60 min at 104±3°C) or rice (45 min at 90±3°C) did not have any influence on the relative weight of the gizzard or affect jejunum digesta viscosity. However, proventriculus weight was larger for birds fed heat treated corn or rice. Further studies by González-Alvarado et al. (2008) showed a smaller

proventriculus size in chick fed stem-cooked (60 min at 104°C) rice compared to unprocessed rice; but no effect was seen in processed corn. In this same experiment, gizzard relative weight was reduced when corn was processed (similar conditions as above), but not when processed rice was fed.

2.8.2 Influence of extrusion on product quality, fiber and nutrient digestibility

Hydrothermal treatments modify the physicochemical structure of the diet, including the fiber component (Bjorck and Asp, 1983), reduce microbes and improve nutrient digestibility (Said, 1996; Mariscal-Landin et al., 2002; Garcia et al., 2008; Al-Marzooqi and Wiseman, 2009; Oryschak et al., 2010b; de Vries et al. 2012). However, excessive heat can denature proteins and decrease their availability (Camire, 1991). Research has shown that heat treatment (e. g., extrusion cooking) alters the physical and chemical properties and results in transformation of insoluble fiber to soluble fiber (Garcias et al., 2008; de Vries et al.., 2012). These soluble fibers form a viscous network by binding water resulting in increased intestinal viscosity (Mateos et al., 2002; Gracia et al., 2003; Scott et al., 2003). Vukic-Vranjes and Wenk (1995) showed a higher (36 g/kg) soluble dietary fiber for extruded barley diet compared to unextruded (28 g/kg). These authors also reported a depression in AME and protein utilization with extrusion. Supplementation of diets with a multi-enzyme complex containing cellulase, β-glucanase and xylanase however restored growth and increased AME (2.9%).

In broilers fed extruded wheat and/or corn DDGS, Oryschak et al. (2010b) reported an increase in the apparent ileal digestibility (AID) of gross energy, crude protein, and amino acids. These diets were fed with an enzyme cocktail which might have contributed to this effect. In a similar experiment by the same authors (Oryschak et al., 2010a), single screw extrusion of triticale DDGS significantly improved the amino acid digestibility in broilers. It should be

understood that ingredients exhibits diverse characteristics during extrusion (Chevanan et al., 2010); and hence modifying processing conditions depends on the type of ingredient used. Knowing how to adapt processing conditions (time, temperature and/or moisture) could be major criteria to ensure beneficial effects of extrusion on particular ingredients in a more consistent manner.

2.9 Wet feeding

Feeding wet diets is a common practice used in the swine industry to enhance nutrient intake and utilization; as well as take advantage of wet ingredients that are available locally. Voluntary feed intake is dependent on the rate of water hydration of feedstuffs which could influence the passage rate and subsequent feed intake (Scott, 2002; Scott and Silversides, 2003). Scott (2002) and Scott and Silversides (2003) demonstrated an improved performance (~20%) of broilers fed wet wheat-based diets compared to dry diets. The increased nutritional value of wet diets might be related to a transformation that occurred in the feed (Forbes, 2003), such as fermentation (Ziemer et al., 2012). Pre-wetting of diets enhances the solubility and allows easy penetration of digestive enzymes (Yasar and Forbes, 2001; Forbes, 2003). More research is required to demonstrate the practical application of wet feeding for industry use.

2.10 Summary and research objectives

Wheat is the predominant feedstock for ethanol production in Western Canada. The interest in the production of ethanol via wheat has resulted in ~1.4 MMT of wheat distillers' dried grain with solubles (Ethanol Producer Magazine, 2013) available as a feed ingredient. Wheat DDGS has been valued as a protein supplement, but its energy content is low due to the fermentation of the starch into ethanol (Cozannet et al., 2009). This co-product has been fed at relatively low levels in poultry diets due to inherent limitations such as higher fiber and concerns about variability in amino acid content and digestibility (i. e., especially lysine; Cozannet et al.,

2009; Noblet et al., 2012). Differences in standardized laboratory procedures to assess the nutritional composition of DDGS, inadequate procedures to quantify sulfur content (Rosentrater, 2012), new techniques in ethanol production (e. g., use of enzymes and oil extraction) and variations in drying process temperatures and duration also contribute to the quality of the final product (DDGS).

Because of an increased supply of DDGS and quality concerns that ultimately impact its utilization to the livestock industry, the value of this co-product for the poultry industry needs to be examined. The industry is currently developing newer technologies to address these issues which will subsequently increase the utility and value of this co-product (Cozannet et al., 2009). The current research evaluated the effects of various levels of wheat DDGS inclusion on turkey hen performance. Moreover, the study investigated if enzymes, extrusion and/or wet feeding positively impact the utilization of wDDGS diets by turkey hens and reduce the limitations of feeding high levels of wheat DDGS by adding value (i.e., improving nutrient digestibility and availability) to the co-product.

3.0 EFFECTS OF WHEAT DISTILLERS DRIED GRAINS WITH SOLUBLES WITH AND/OR WITHOUT PROTEASE AND β-MANNANASE ON THE PERFORMANCE OF TURKEY HEN POULTS

3.1 Abstract

Expansion in bioethanol production has resulted in distillers dried grains with solubles (DDGS) being readily available as a major protein source in the poultry industry. Two experiments were conducted to investigate effects of wheat DDGS (wDDGS) and enzyme on nutrient digestibility and performance of turkey hen poults (7-21d). Two starter diets (0 or 30% wDDGS) were formulated to meet or exceed the nutrient requirements for Hybrid Converter female turkeys. These diets were then mixed in different proportions to obtain two additional wDDGS inclusion levels (10 and 20%). In experiment 1, 0 and 30% wDDGS diets were each sub-divided into 3 portions and supplemented with no enzyme (E-), protease (P+; 0.125 g/kg) or β-mannanase (M+; 0.5 g/kg). A total of 144, 7d old poults were randomly distributed in groups of 4 in 6 replicate cages per treatment. There were no significant main effects or interactions on feed intake from 7 to 21d. However, a positive ($P<0.05$) effect of 30% wDDGS was shown for 21d body weight (BW) and feed conversion ratio (FCR). There were no significant main effects of enzymes or wDDGS level on nitrogen retention (NR); however a significant interaction on NR was found. There were significant main effects and interactions on the AME of the diets. The AME was higher ($P<0.05$) for 30% compared to 0% wDDGS. Supplementation of P+ decreased ($P<0.05$) AME for 0% diets as compared to 30% diets and vice versa for M+. In experiment 2, 7 d old poults (4 birds per 6 replications per treatment) were fed 4 levels of wDDGS (0, 10, 20 and 30%) with no enzyme. A quadratic ($P<0.01$) response was found for FCR, with 30% wDDGS having the highest value. Quadratic ($P<0.01$) responses were also found for NR and AME; both were highest for 10% wDDGS diets. In summary, no beneficial effects of P+ or M+ were

demonstrated in diets containing 30% wDDGS. Wheat DDGS is a valuable energy source and as high as 30% can be incorporated in turkey hen poults diets.

3.2 Introduction

Ethanol as a biofuel has a comparative advantage over petroleum gas because of its production sustainability, increased energy level and lower combustion emissions (Lumpkins et al., 2004; Swiatkiewicz and Koreleski, 2008). Distillers dried grains with solubles (DDGS) are co-products produced by fermentation of starch from cereals for ethanol production (Pahm et al., 2008; Cozzanet et al., 2011). The quality of DDGS is dependent on the grain source, extraction process and drying conditions, and may be highly variable in nutrient level and digestibility (Fastinger et al., 2006). Distillers dried grains with solubles are predominantly used in ruminant diets due to high fiber content (Cozzanet et al., 2010; Abdel-Raheem et al., 2011). Fiber levels are one of the concerns with respect to use of DDGS in poultry diets due to the dilution of the diet and potential antinutritive factors (Emiola et al., 2009). However, DDGS contain high levels of amino acids and reasonable levels of energy (Nyachoti et al., 2005; Kluth and Rutherford, 2010; Abdel-Raheem et al., 2011), and therefore have potential as a feedstuff for poultry and other non-ruminants (Bolarinwa and Adeola, 2012).

The bioethanol industry utilizes different sources of cereal grains for ethanol production. In Canada and Western Europe wheat is the major cereal grain used for ethanol production (Cozzanet et al., 2010), whereas corn is predominant in USA (Fastinger et al., 2006; Noll and Brannon, 2006). There is some research on the use of wDDGS in chicken diets (Nyachoti et al., 2005; Thacker and Widyaratne, 2006; Bolarinwa and Adeola, 2012), but little reference on the use of wDDGS in turkey diets. In general, these studies have shown that wDDGS has low energy but that performance was not negatively affected when alternative sources of energy were

added. Low energy in DDGS is due to the conversion of the starch to alcohol in the ethanol production process and increasingly the pre-harvesting of oil, particularly with corn as a substrate for biodiesel production.

Although wheat DDGS contain a high level of protein, there are concerns about the level and digestibility of amino acids, most especially lysine (Cozzanet et al., 2010; Bolarinwa and Adeolu, 2012). Research has shown that by keeping the energy level constant, levels as high as 25% DDGS can be incorporated into poultry diets without detrimental effects (Waldroup et al., 1981). Nonetheless, DDGS was traditionally held at about 5% inclusion levels in commercial poultry diets (Lumpkins et al., 2004). Although higher inclusions of DDGS have been investigated in pig diets over the years (Emiola et al., 2009), inclusion of DDGS as high as 30% have not been investigated in poultry diets. Therefore, investigating the incorporation of DDGS at higher levels in poultry production will be an added advantage to the industry (Noll et al., 2001). In the present study, it was also expected that higher levels of wDDGS would facilitate the evaluation of enzymes to improve nutrient availability (Bolarinwa and Adeola, 2012).

Since DDGS contain approximately 6% yeast biomass, which is rich in mannan, there may be antinutritional effects associated with mannans (Radfar et al., 2013). Enzymes can affect the nutritional quality DDGS (Omogbenigun et al., 2004; Nyachoti et al., 2006; Emiola et al., 2009). However, the enzymes used in these studies differ from the current study. Supplementation of enzymes (e.g., proteases or β-mannanase) to low quality feed ingredients such as wDDGS, may improve their nutritional value for poultry. The objective of the current research was to determine the effect of increasing levels of wDDGS, and protease and β-mannanase supplementation on nutrient availability and performance of turkey hen poults.

3.3 Materials and methods

All experimental protocols and procedures were approved by the Animal Care Committee (animal use protocol no. 19940248) of the University of Saskatchewan and care of the birds was in accordance with the recommendations of the Canadian Council of Animal Care (1993).

3.3.1 Diets formulation and assay diets

The wDDGS used in the current study was obtained from a local ethanol processing plant (Husky Lloydminster, Saskatchewan, Canada). Two basal diets containing 0 or 30% wDDGS (Table 3.1) were formulated to meet or exceed the nutrient requirements of Hybrid Converter turkey starter diets (http://www.hybridturkeys.com/hybrid-resources/nutritional-guidelines). Both diets were mash and were formulated (Cargill Animal Nutrition, North Battleford, Saskatchewan) to be isonitrogenous (28% CP) and isocaloric (2760 kcal ME/kg). The final test diets were mixed at the University of Saskatchewan feed mixing facility. Poults had free access to a commercial wheat-soybean turkey starter diet (crumble; Co-op Feeds, Saskatoon) providing 28.5% CP, 2780 kcal ME/kg, 0.77% methionine, 1.84% lysine, 1.65% calcium and 1.18% total phosphorus until 7d of age.

3.3.1.1 Experiment 1

The 0% and 30% wDDGS based diets were partitioned into three portions and then supplemented with no-enzyme (E-), protease (P+; 0.125g/kg) or β-mannanase (M+; 0.5g/kg). The enzymes were supplied by Jefo Nutrition Inc. (5020 Avenue Jefo, C.P. 325, St-Hyacinthe, Québec, Canada). The six diets tested in experiment 1 were 0% wDDGS (E-, P+ or M+) and 30%wDDGS (E-, P+ or M+).

Table 3.1 Composition of experimental diet (Experiments 1 and 2). In experiment 1, diets were fed as is or supplemented with either protease (0.125 g/kg) or β-mannanase (0.5 g/kg). In experiment 2, the two diets were blended to provide 0, 10, 20 and 30% wheat DDGS (wDDGS) inclusion levels (no enzymes were fed in these diets)

Item	0wDDGS (%)	30wDDGS (%)
Ingredient		
Wheat DDGS	0.000	30.000
Wheat	45.700	15.100
Corn	0.000	14.600
Soybean meal	20.500	15.600
Canola meal	9.320	0.000
Porksoya[a]	18.000	18.000
Fat	2.790	3.000
Dicalcium phosphate.	1.620	1.200
Trace minerals premix[b]	0.075	0.075
Calcium carbonate	0.811	1.226
Salt	0.241	0.289
Broiler vitamin premix[c]	0.060	0.060
Choline chloride (60%)	0.075	0.075
DL-Methionine	0.188	0.179
L-Lysine HCL	0.000	0.564
Vitamin D - HyD	0.069	0.069
Sodium sesquicarbonate	0.040	0.188
Indigestible marker (celite)	0.500	0.500
Calculated nutrient		
AME (kcal/kg)	2760	2760
Crude protein (%)	28.00	28.00
Fat (%)	6.00	8.43
Ash (%)	7.72	7.55
Fiber (%)	3.29	3.80
Calcium (%)	1.50	1.50
Total phosphorus (%)	1.14	1.03
Lysine (%)	1.73	1.75
Methionine (%)	0.62	0.65
Threonine (%)	0.98	1.01
Met + Cys (%)	1.12	1.14

[a]ME, 3006 kcal/kg; Protein, 51.6%; fat, 10.9%; fiber, 3.86%; calcium, 4.40%; phosphorus, 2.42; potassium, 1.53; sodium, 0.30; arginine, 7.37%; histidine, 2.31%; isoleucine, 4.00; leucine, 7.42; lysine, 5.98; methionine, 1.52; phenylalanine, 4.33%; threonine, 3.78; tyrosine, 1.06; valine, 5.02%.

wDDGS=Wheat distillers dried grains with solubles

[b] Supplied per g or kg of diet: iron, 120 mg/kg; zinc, 117 mg/kg; manganese, 110 mg/kg; copper, 22 mg/kg; iodine, 1.5 mg/kg; selenium, 0.3 mg/kg

[c] Supplied per g or kg of diet: vitamin A (retinyl acetate + retinyl palmitate), 14.1 IU/g; vitamin D, 3.90 IU/g; vitamin E (dl-α-topheryl acetate), 42 IU/kg; thiamine, 3.0 mg/kg; riboflavin, 8.4mg/kg; niacin, 60mg/kg; vitamin B_6, 6.0 mg/kg; vitamin K, 3.60mg/kg; vitamin B_{12}, 0.02 mg/kg; pantothenic acid,18 mg/kg; folic acid, 1.32mg/kg; biotin, 0.18 mg/kg

3.3.1.2 Experiment 2

The two basal diets (0% and 30% wDDGS) were proportionally mixed to obtain four different inclusion levels (0%, 10%, 20%, and 30% wDDGS); no enzymes were used in these diets.

3.3.2 Experimental birds and management

A total of 192 day old Hybrid Converter turkey hens (Lilydale Hatchery, Edmonton, Alberta) were placed in battery cages at the University of Saskatchewan Poultry Centre. Birds were maintained in groups of 12 for the first 7 d. On d 7, birds were wing banded and individually weighed and assigned to respective treatment groups. Turkey poults were provided free access to feed and water. Room brooding temperature was 32°C at 0 d and was then gradually reduced to 23°C at 21 d. Birds had 18 h of light and 6 h of dark with a light intensity of 10-20 lux.

3.3.2.1 Experiment 1

In experiment 1, four poults (7 d of age) were randomly allocated to each of 36 battery cages measuring 29.2 cm (height) × 48.3 cm (depth) × 83.8 cm (width) and providing 1010 cm^2/bird. Cage served as a replicate and there were six replicates per treatment. Cages were randomly assigned to one of six dietary treatments [0% wDDGS (E-, P+ or M+), 30%wDDGS (E-, P+ or M+)] from 7 to 21 d.

Body weight was recorded on d 7, 14 and 21, as was feed intake for corresponding periods of time. Feed conversion ratio (FCR) corrected for mortality was calculated. For apparent metabolizable energy (AME) and nitrogen retention (NR) determination, excreta were collected four times between 19-21 d with plastic sheets laid on trays under the battery cages. Clean (free of feathers and feed) excreta samples were frozen (-20C) until analyzed.

At the end of the trial (21 d) all four birds in each replicate cage were humanely killed by cervical dislocation and their digestive tract (gut) segments were removed. The weights of empty fat-free gizzard (i. e., fat removed around the gizzard) and proventriculus, and the lengths of the duodenum (intestinal segment directly associated with pancreas), jejunum (from distal duodenal loop to Meckel's diverticulum), ileum (Meckel's diverticulum to ileal-cecal junction); and total ceca were recorded. All weight and length measurements of the gut segments were expressed relative (%) to the body weight of the individual bird.

3.3.2.2 Experiment 2

In experiment 2, 7 d old turkey poults were randomly allocated to 24 battery cages as described in experiment 1. Poults were assigned to four different dietary treatments (0, 10, 20, and 30% wDDGS), with four birds per cage and six replicates per treatment. Performance and gut segment measurements made in experiment 2 were the same as in experiment 1.

3.3.3 Chemical analysis

The excreta samples collected in experiments 1 and 2 were oven dried for 72 h at 55°C for dry matter determination. After drying, samples from each replicate were pooled together for analysis. Both diet and excreta was ground using a Retsch grinder with a 1.0 mm screen (ZM-100, Rheinische Strabe 36 D-42781 Haan, Germany). All analyses were done in duplicate. Dry matter was determined by drying in a forced-air oven at 135°C for two h (AOAC 15thed, 1990). Crude protein (N × 6.25) was determined using a Leco analyzer (Model FP-528L, Leco Corp. St. Joseph MI, USA) and EDTA as a standard, according to the procedure described in AOAC (1995). Gross energy was determined by adiabatic oxygen bomb calorimeter (PARR, 1281, Moline, Illinois, USA), using benzoic acid as a standard. Celite^{TM}585 (Acros Organic, Fisher Scientific), an acid insoluble ash marker (AIA), was analyzed using a modified procedure from

Vogtmann et al. (1975). To measure AIA, 1-2 g of samples was weighed into 16×125 mm glass tubes (VWR North America, West Chester, PA, USA). The tubes were heated at 500°C for 24 h. The ash samples were then mixed with 5 mL of 4 N HCl and then oven heated for 1 h at 120°C. Samples were then centrifuged at 2500 rpm for 10 min. The supernatants were carefully removed using a vacuum siphon and samples washed twice with 5 mL water and then dried at 80°C overnight. These dried samples were further kilned at 500°C overnight.

3.3.4 Calculations

Calculation of apparent metabolizable energy (AME) and nitrogen retention (NR) were based on those used by Scott and Hall (1998). The formulas used for the calculation are below.

$$AME\ (kcal/kg\ of\ diet) = GE_{diet} - [GE_{excreta} \times (Marker_{diet}/Marker_{excreta})]$$

$$NR = 100 - [100 \times (\%Maker_{diet}/\%Marker_{excreta}) \times (\%N_{excreta}/N_{diet})]$$

3.3.5 Statistical analysis

In experiment 1, the data were analyzed as a 2×3 factorial arrangement using Proc GLM (General Linear Model) of SAS version 9.2 (SAS Institute Inc,1996). There were two levels of wDDGS (0 or 30%) and three enzyme [none (E-), protease (P+) and β-mannanase (M+)] treatments. Differences were considered statistically significant when $P \leq 0.05$. Duncan's Multiple Range Test was used for mean separation when the statistical analysis was significant.

In experiment 2, the different inclusion levels (0, 10, 20, and 30%) were used to demonstrate the effect of increasing levels of wDDGS in the starter diets of turkey hens. Regression analysis [ProcReg and RSReg of SAS 9.2 (SAS Institute Inc, 1996)] was used to measure linear and quadratic responses. Data were considered significant when $P \leq 0.05$

3.4 Results

The chemical composition of the wDDGS used in the diet formulation was 35.9% CP, 93.3% DM, 4.57% fat and 7.22% crude fiber. Analyzed nutrient compositions of the respective dietary treatments are shown in Table 3.2. There was no mortality recorded during either experiment.

Table 3.2 Analyzed nutrient composition of dietary treatments fed to determine the effects of wDDGS on turkey hen poults (as fed)

Item	Dry Matter (%)	AME (kcal/kg)	Protein (%)
Experiment 1			
0% wDDGS	91.2	2741	27.0
30% wDDGS	92.2	2972	28.7
0%wDDGS+ P	91.1	2854	28.7
0%wDDGS +M	91.0	2970	25.6
30%wDDGS +P	92.2	2965	26.6
30%wDDGS+ M	92.2	2858	28.5
Experiment 2			
0% wDDGS	91.2	2741	27.0
10% wDDGS	91.4	3004	26.6
20% wDDGS	92.8	2933	28.0
30% wDDGS	92.2	2972	28.7

AME = apparent metabolizable energy
wDDGS =wheat distillers dried grains with solubles
P = Protease (0.125 g/kg)
M = β-mannanase (0.5 g/kg)

3.4.1 Experiment 1

The means for 21 d body weight (BW), feed intake (FI; 7-21 d) and FCR (7-21 d) are presented in Table 3.3. There were no effects of treatment on 7 d BW (149 ± 4.0g). At 21 d, BW was significantly higher for poults fed diets with 30% wDDGS, but enzyme treatment had no effect. There were no treatment effects on FI. Poults fed 30% wDDGS had a lower FCR value than those fed 0% wDDGS, while no differences were found due to enzyme treatment.

Table 3.3 Experiment 1. Effects of wheat distillers dried grains with solubles (0 or 30% wDDGS) with and without protease (0.125 g/kg) or β-mannanase (0.5 g/kg) during the starter phase (7-21d) on mean 21 d body weight, feed intake and feed conversion ratio of turkey hen poults

Item	*df* n	Body weight (g/b)	Feed intake (g/b/d)	Feed conversion ratio (g/g)
Level of wDDGS	*1*	*	NS	*
0% wDDGS	18	651	52.3	1.50
30% wDDGS	18	680	52.3	1.41
Enzymes	*2*	NS	NS	NS
None	12	675	53.0	1.46
Protease	12	664	52.3	1.46
β-Mannanase	12	657	51.6	1.45
wDDGS Level*Enzyme	*2*	NS	NS	NS
SEM		13.5	1.07	0.016

SEM=Standard error of means.
*$P<0.05$

Nitrogen retention was not influenced by level of wDDGS or enzyme treatment (Table 3.4), but there was a significant interaction between them (Figure 3.1). Nitrogen retention for birds fed 0% wDDGS was higher for the β-mannanase treatment than for either the un-supplemented or protease treatments. Whereas, with 30% wDDGS, NR for the β-mannanase treatment was numerically and significantly lower than the un-supplemented and protease treatments, respectively. The AME of the 30% wDDGS treatment (Table 3.4) was higher than for the 0% wDDGS inclusion level. There was the tendency (P=0.09) for β-mannanase supplementation to improve AME as compared to no enzyme and protease treatments.

Table 3.4 Experiment 1. The effects of wheat distillers dried grains with solubles (0 or 30% wDDGS) with and without protease (0.125 g/kg) or β-mannanase (0.5 g/kg) on nitrogen retention and apparent metabolizable energy of turkey hen poults

Item	Nitrogen retention (%)	AME (kcal/kg)
Level of wDDGS	NS	*
0% wDDGS	53.1	2855
30% wDDGS	51.9	2932
Enzymes	NS	P=0.09
None	51.6	2857
Protease	52.8	2910
β-mannanase	53.4	2914
wDDGS Level*Enzyme	*	*
SEM	1.38	27.7

SEM=Standard error of means.
*$P<0.05$

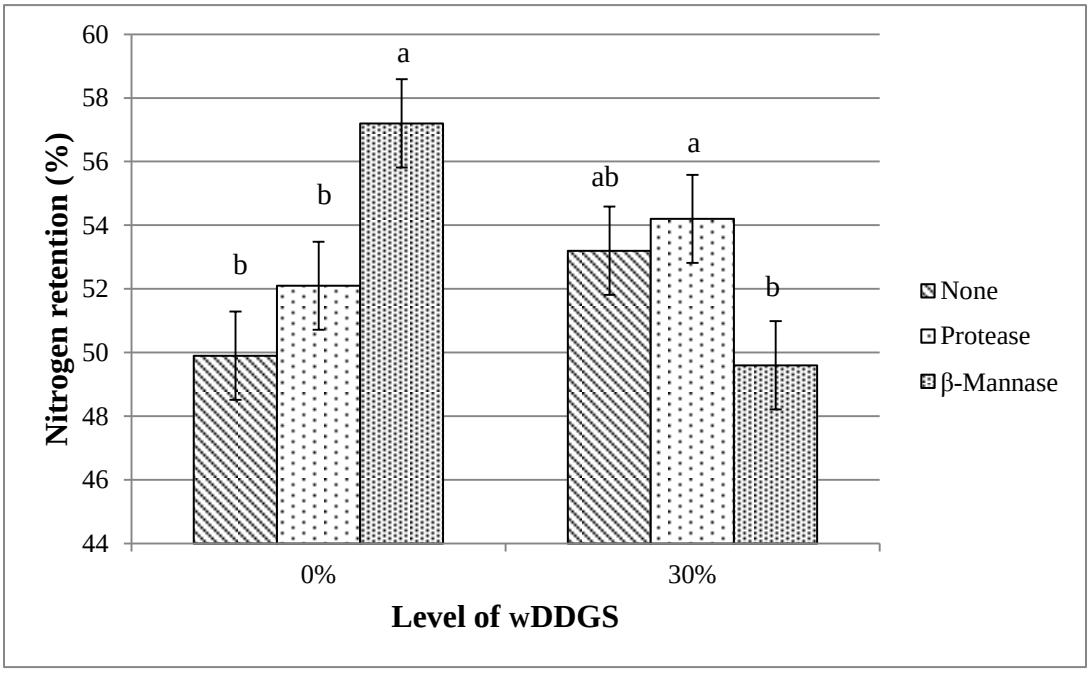

Figure 3.1.: Experiment 1. The interaction between wheat distillers dried grains with solubles (wDDGS) levels and enzyme (none, protease or b-mannanase) on nitrogen retention (%).
Bars without common letters (a, b) are significantly different (P<0.05)

An interaction was found between enzyme use and level of wDDGS on the AME of diets (Figure 3.2). With 0% wDDGS, both protease and β-mannanase treatments increased AME, and the improvement was greater for β-mannanase than for protease. Whereas, at 30% wDDGS, the

AME value for the protease treatment was equal to the un-supplemented treatment and the β-mannanase value was lower than the other two 30% diets.

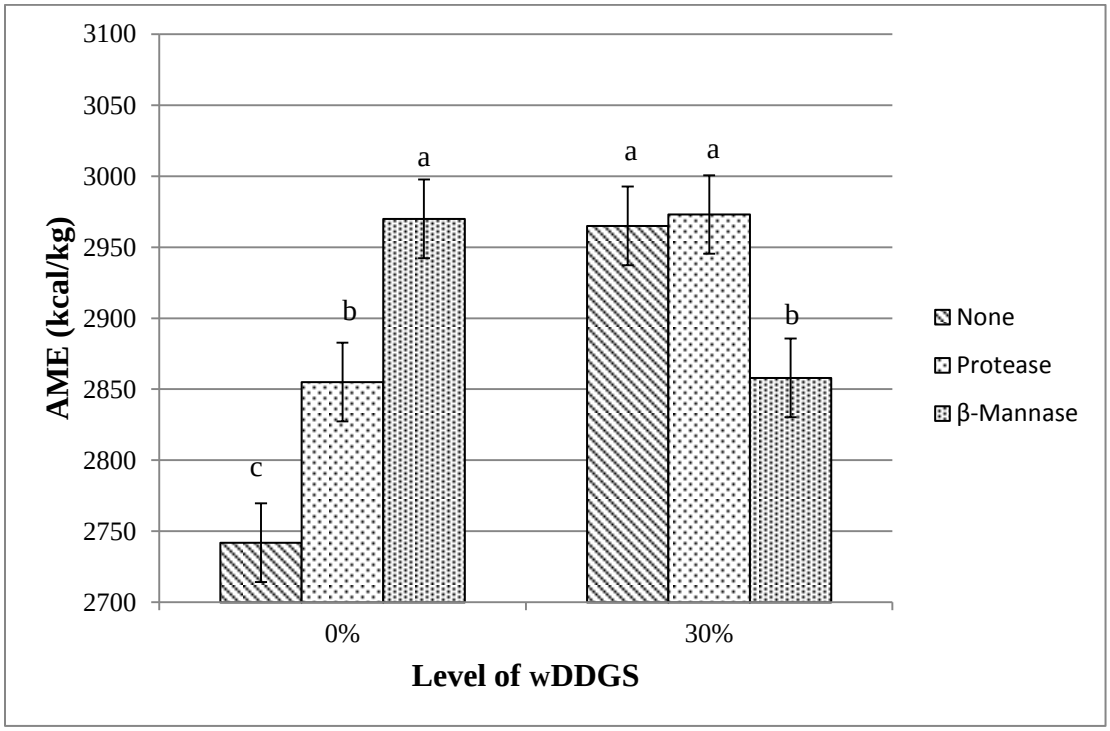

Figure 3.2. Experiment 1. The interaction between wDDGS levels and enzymes on apparent metabolizable energy. Bars without common letters (a-c) are significantly different (P<0.05)

There were no main or interaction effects for relative measurements of gut segments (data not shown). The main effect of level of wDDGS inclusion on ileal length, however, tended to be higher (4.8%) (P=0.06) for the 0% inclusion than the 30%.

3.4.2 Experiment 2

The effects of inclusion level of wDDGS (no enzyme) on BW, FI and FCR are shown in Table 3.5. There was no effect of wDDGS inclusion level on BW and FI (7-21 d). There was a significant quadratic relationship (P<0.05) between level of wDDGS and FCR (7-21 d), with 30% inclusion having the lowest FCR.

Table 3.5. Experiment 2. Effects of wheat distillers dried grains (0, 10, 20 or 30%; wDDGS) with solubles during the starter phase (7d -21d) on body weight, feed intake and feed conversion ratio (FCR) of turkey hen poults

Item Level of wDDGS inclusion	Body weight (g/b)	Feed intake (g/b/d)	FCR (g/g)
0% wDDGS	644	51.7	1.51
10% wDDGS	667	52.6	1.45
20% wDDGS	662	51.7	1.44
30% wDDGS	671	51.6	1.42
SEM	15.5	1.36	0.015
P-Value	NS	NS	*
Equation	-	-	$Y=1.50 - 0.00053x + 0.000086x^2$

SEM=Standard error of means.
NS=not significant
*Quadratic regression with $P < 0.05$.
Y = FCR
X = inclusion level of wDDGS

The relationships between wDDGS inclusion level and NR and AME are summarized in Table 3.6. Both linear and quadratic relationships ($P<0.01$) were found for NR with the highest NR occurring with 10% and the lowest with 0% wDDGS inclusion. Linear and quadratic relationships were also found for AME, with the lowest and highest values for the 0 and 10% inclusion levels, respectively. Overall both NR and AME values for the 10, 20 and 30% inclusion were higher (approximately 11 and 8%, respectively for NR and AME) than the 0% inclusion.

Table 3.6. Experiment 2. The effects of dietary inclusion (0, 10, 20 or 30%) wheat distillers dried grains with solubles (wDDGS) on excreta nitrogen, nitrogen retention and apparent metabolizable energy (AME) of turkey hen poults (21 d of age)

Item	Nitrogen retention (%)	AME (kcal/kg)
Level of wDDGS inclusion		
0% wDDGS	49.9	2742
10%wDDGS	58.6	3004
20%wDDGS	54.3	2933
30%wDDGS	53.2	2973
SEM	1.27	35.8
P-Value	**	**
Equation	$Y = 50.7 + 0.793x - 0.025x^2$	$Y= 2763 + 22.9x - 0.557x^2$

SEM=Standard error of means
NS=not significant
**Quadratic regression with $P < 0.01$
Y = AME/Nitrogen retention
X = inclusion level of wDDGS

There was no effect of wDDGS inclusion level on the relative length of duodenum, jejunum, ileum or caeca (data not shown). However, a quadratic response was found between inclusion level of wDDGS and relative proventriculus weight; the highest was for 10% inclusion level ($P \leq 0.05$; $Y=0.466 + 0.004x - 0.0001x^2$). There was also the tendency for inclusion levels of wDDGS to affect relative gizzard weight in a quadratic fashion ($P = 0.07$; $Y = 2.43 + 0.020x - 0.0006x^2$); in this instance 20% wDDGS inclusion resulted in the highest value.

3.5 Discussion

The objective was to determine the nutrient digestibility and the performance of turkey hen poults fed wDDGS with and/or without protease or β-mannanase. If enzymes can increase the value of wDDGS by improving the availability and nutrient consistency, then using higher levels such as 30% may assist in demonstrating this effect.

The Hybrid Converter turkey guideline indicates that average BW of turkey hens at 21 d is expected to be 0.76 kg. In the current study, the average BW of birds ranged between 0.65-

0.68 kg for all the dietary treatments. This is approximately 11.8-16.9% less for BW than Hybrid Converter performance goals. This value (including the 0% wDDGS treatment) indicates that the poults were underperforming as compared to expected performance standards. Low performance might be associated with health or environmental restraints, however, no mortality was observed in either experiment during the study period and monitored environmental conditions were similar to recommended values. It may also relate to a common ingredient(s) in the diets with and without wDDGS that caused the reduced performance.

Yoon et al. (2011) reported that mannan can negatively affect growth performance of animals. The use β-mannanase has improved performance of animals fed soybean-based diets (Odetallah et al., 2002; Pettey et al., 2002; Mehri et al., 2010; Jackson et al., 2008). However, there are no studies that have evaluated the effect β-mannanase use in wDDGS -based diets. In the present study, no effect of β-mannanase was noted in diets with 0 or 30% wDDGS. Vahjen et al. (2005) similarly reported no improvement in performance when a combination of β-mannanase and galactanase was supplemented in a soybean-based diet. However, Jackson et al. (2008) reported an improvement in FCR when DDGS supplemented with β-mannanase was fed to turkeys. Other researchers have also shown a significant improvement in broilers fed a corn-soybean meal diet supplemented with β-mannanase, protease and/or a combination of protease and xylanase (Kong et al., 2011; Kalmendal and Tauson, 2012).

From a practical perspective, the efficacy of enzymes can be demonstrated when supplemented in diets containing low digestible ingredients compared to highly digestible ingredients (Bedford, 2000). Soybean meal, which is a highly digestible ingredient, was at a higher rate in the 0% wDDGS as compared to the 30% wDDGS. This may explain why protease was not effective in the 0% wDDGS on performance, but not why it was ineffective in diets

containing 30% wDDGS. Therefore, the hypothesis that enzymes could improve the nutritive value of low digestible ingredient such as wDDGS at 30% inclusion failed in the current experiment and requires further investigation and an evaluation of variability between sources of wDDGS and enzymes.

Abdel-Raheem et al. (2011) reported negative effects on broiler performance when DDGS (wheat-corn) was fed at increasing levels (0, 6 and 12%). Although feed intake from 7-21 d was not affected by treatment in experiment 1, there was an improvement in 21 d BW and FCR for bird fed 30% wDDGS. This may be due to the 30% wDDGS diet containing higher energy, which was found to be the case (Table 3.5). However, the differences in results could be attributed to the digestive efficiency for fiber by different avian species. It was reported by Duke (1996) that turkeys, compared to other avian species, have a capacity to digest fibrous feed ingredients. There is also further work required to understand the impact of ethanol production on the physicochemical properties of fiber in grain and how this impacts commercial poultry.

In experiment 2, all levels of dietary wDDGS (10, 20, and 30%) showed an improvement in FCR compared to 0% inclusion, but other performance indices were not affected. Wang et al. (2007) found no negative impact of feeding 20% corn DDGS to broilers, but at 25% inclusion, there was higher FI. Similar results with no negative influence were also reported by Xu et al. (2007) when corn DDGS was fed at 30% to pigs. Lineen et al. (2008) reported decreased performance when 20% corn DDGS was included in pig diets.

Improvements in nutrient digestibility are usually related to the decrease in the fiber content of a diet (Thacker et al., 2013). One major limitation of DDGS is its high dietary fiber causing a reduction in nutrient digestibility (Spiehs et al., 2002; Thacker and Widyaratne; 2007; Jimenez-Moreno et al., 2009) due to its physiochemical properties, leading to nutrient dilution

which can either decrease the passage rate in the upper digestive tract (Hetland et al., 2005) or increase passage rate in the lower digestive tract limiting enzymatic activities and compromising the absorption of nutrients. Another concern that limits inclusion of DDGS as a feed ingredient is the high variability of nutrient inherent in various DDGS sources (Liu, 2012). As a consequence, nutritionists will maintain a higher margin of error in using these ingredients. This process can lead to imbalances in the diet. The higher inclusion of fat to the 30% inclusion might have resulted in an increase energy compared to the 0% inclusion that was formulated with ingredients containing less energy than anticipated. There is also a possibility of a higher level of available energy (i.e., non-fermented starch) in the particular source of wDDGS fed in the present study.

There are other sources of variability in production of DDGS, including cereal type, cereal processing before fermentation (i.e., removal of oil from corn), fermentation method, processing conditions of DDGS (e.g., temperature and time), and amount of solubles returned to the DDGS (Spiehs et al., 2002; Fastinger et al., 2006; Martinez-Amezcua, 2007; Pahm et al., 2008; Abdel-Raheem et al., 2011; Bolarinwa and Adeola, 2012). Based on this, there may have been higher levels of starch or sugars in the sample of wDDGS used in the present study. Unfortunately, starch level and processing conditions of the wDDGS are not available in the current experiment and cannot be used to explain the research results such as the improvement in AME for 30% wDDGS in experiments 1 or 2.

A quadratic response in AME as level of wDDGS increased was found in experiment 2 with the highest value at 10% inclusion. This would suggest that at higher levels of inclusion the birds were not able to retain energy or nitrogen as well. The results in the current experiment is

in agreement with Bolarinwa and Adeola (2012) who showed a decrease in energy digestibility as levels of wDDGS increased in broiler chickens.

Although performance was not affected with wDDGS inclusion, Emiola et al. (2009) showed significantly better performance for pigs fed diets supplemented with a carbohydrase enzyme source that supplied 2200 U of xylanase, 1100 U of β-glucannase and 1200 U of cellulase. A lack of enzyme response in the present study may relate to an inappropriate choice of enzyme suitable for the types of substrate in the diet (Emiola et al., 2009).

An improvement in total tract digestibility of energy and nitrogen was seen in a corn-soybean diet supplemented with β-mannanase (Zou et al., 2006; Kong et al., 2011). Kong et al. (2011) reported a tendency (P=0.06) towards improved nitrogen utilization with β-mannanase supplementation. Yoon et al. (2011) on the other hand, showed a significant increase in apparent total tract digestibility of crude protein when β-mannanase was supplemented in a corn DDGS diet fed to pigs. Additionally, a positive response to β-mannanase and protease may be dose dependent that may not have matched the supplementation level used in this research. Enzymes were added at recommended levels (β-mannanase was added at 0.05g/kg and protease at 0.125g/kg) to both the 0 and 30% wDDGS diets.

The significant interaction in digestibility was not reflected in performance, which requires further investigation. The interaction in digestibility may relate to different levels of mannan in the two diets due to differences in soybean inclusion or to mannans from yeast cell wall fractions in DDGS. Mannan is a major NSP in soybean (1.3 to 1.6%; Jackson et al., 1999; Mehri et al., 2010). The 0% wDDGS diets had higher levels of soybean as compared to 30% wDDGS diets; therefore this may explain the higher response due to β-mannanase when included

in 0% wDDGS diets. Further study is needed to explain the reason for the reduction in both NR and AME with β-mannanase supplementation on 30% wDDGS.

Despite the higher inclusion of wDDGS in experiment 1, and expected higher levels of fiber, there was no increase in gizzard weight. This could be related to particle size (finely ground particles) of the dietary treatment; however, particle size analysis was not done. Similar results in terms of gizzard weight were shown by Abdel-Raheem et al. (2011), who recorded no difference in absolute gizzard weight of broilers fed graded levels of DDGS. The gizzard weight in experiment 2 was higher at 20% and reduced at 30% inclusion. This could be due to the fact that, the process of fermentation has changed the physiological effect of fiber on the gut; hence the bird did not respond to increased fiber by increasing size of the gut segments. It may also be speculated that gut fill was a limiting factor and that poults were not able to adapt in the 7 to 21 d period. The quadratic response that was seen with proventriculus weight in experiment 2 (data not shown), which was higher at 10% inclusion (10>20>30) calls for further investigation. There was the possibility that, the increasing levels of wDDGS resulted in increased residence time of digesta in the upper gut and exposed to more complete acid and enzyme digestion. But because no differences were recorded in gut size and a decrease of nutrient digestibility at increasing levels, it is difficult to explain.

This research has indicated that wDDGS is a suitable ingredient to be considered for poults; hence by adapting to precise formulation of diets using wDDGS, as high as 30% can be incorporated in turkey starter diets. However, in terms of performance, the results failed to demonstrate any effect of protease or β-mannanase on either the 0 or 30% wDDGS diets. It could also be of interest to set up an experiment to investigate the use of an enzyme cocktail (i. e., with a wide range of activity) and their efficacy at various inclusion levels of wDDGS.

4.0 EVALUATION OF INCLUSION LEVEL OF WHEAT DISTILLERS DRIED GRAINS WITH SOLUBLES WITH AND/OR WITHOUT PROTEASE OR β-MANNANASE ON PERFORMANCE AND WATER INTAKE OF TURKEY HENS

4.1 Abstract

It is becoming a common practice to use higher levels of wheat distillers dried grains with solubles (wDDGS) in poultry diets. The objective of this experiment was to determine the effects of level of inclusion of wDDGS with or without enzyme supplementation on performance and water consumption on turkey hens (0-72 d). Two diets (0 or 30% wDDGS) were formulated to meet the nutrient requirements of Hybrid Converter turkeys. Diets (starter, grower and finisher) were then blended to obtain different levels of inclusion (0, 15 or 30%) of wDDGS for each feeding phase. The 30% wDDGS diet was divided into 3 fractions and 2 fractions supplemented with either protease (P+; 0.126 g/kg) or β-mannanase (M+; 0.05g/kg). All five diets were fed ad libitum as mash. The 700 0 d turkey hens were randomly allocated into groups of 35 birds per replicate with 4 replicate floor pens per treatment, in a completely randomized design. Water consumption per pen was recorded beginning at 7 d. There was no effect of dietary treatment on FI. Body weight of turkey hens (52 d; grower) was significantly higher for 30% wDDGSP+ as compared to 0% wDDGSE- or 15% wDDGSE- diets; but was not different from 30% wDDGSE- or 30% wDDGSM+ diets. The FCR ($P<0.01$; 28-52 d), and total FCR ($P<0.05$; 0-72d) was significantly improved for birds fed 30% wDDGS regardless of enzyme treatment compared to 0% wDDGSE- and 15% wDDGSE- diets. Water intake (mL/b/d) tended to be higher ($P=0.08$) between 7-28 d for 30% wDDGSP+ diets. Similarly, water intake of birds fed 30% wDDGSP+ was higher ($P<0.05$; 28-52 and 52-72 d) and total water intake ($P=0.07$; 7-72 d) tended to be higher than other treatments. To our knowledge, this experiment is the first to report the impact of wDDGS on water intake. As high as 30% wDDGS can be substituted in

turkey hen diets. No effect of protease or β-mannanase addition at the inclusion level tested was found on performance.

4.2 Introduction

Fuel ethanol production from cereal grain has significantly increased and evolved since the early 1990's, using a fermentation process that is considerably different from beverage-alcohol production (Bregendahl, 2008; Ganesan et al., 2007). Ethanol production from cereals claims an advantage over regular gasoline, by reducing greenhouse gas emission (Świątkiewicz et al., 2013). The increase in ethanol production, and it's improved consistency, has resulted in a substantial quantity of distillers dried grains with solubles (DDGS) as a feed ingredient for monogastrics (Chevanan et al., 2010; Thacker and Widyaratne., 2007; Bregendahl, 2008; Świątkiewicz and Koreleski, 2007; Lim et al., 2009; Oryschak et al., 2010; Fallahi et al., 2010).

Traditionally, the recommendations for inclusion of DDGS in poultry diets was low due to limitations in supply and/or variability in nutritional composition (Noll et al., 2001; Martinez-Amezcua et al., 2007; Swiątkiewicz and Koreleski, 2007; Waldroup et al., 2007; Leytem et al., 2008; Olokosi et al., 2010). Inclusion levels of 5-15% in laying hens (Lumpkins et al., 2005; Shalash et al., 2010), 5-30% in broilers (Thacker and Widyaratne, 2007; Wang et al., 2007; Min et al., 2008; Cozannet et al., 2010; Oryschak et al., 2010) and 10-20% in turkey diets (Roberson, 2003; Noll and Brannon, 2006) have been reported. These studies used different sources of DDGS produced from corn, wheat and/or triticale. Lumpkins et al. (2005) showed improved egg production, egg weight, yolk color, exterior and interior egg quality by feeding corn DDGS at 15% inclusion to laying hens. Min et al. (2008) also reported that up to 30% corn DDGS can be incorporated in broiler diets without any adverse effect on production. Leytem et al. (2008) fed wheat DDGS to broilers in the ratio of 5, 10, 15 or 20% and reported an increase in nutrient digestibility at increasing levels. Like many other co-products, there are concerns with regards

to variations in dietary energy, bioavailability of lysine and high fiber (Waldroup et al., 2007; Noblet et al., 2012; Ziemer et al., 2012).

Enzymes are increasingly being used to reduce antinutritional factors in feed ingredients (Wu et al., 2005; Cowieson et al., 2006). Wheat DDGS contains yeast cell wall mannan and therefore, it may benefit from carbohydrase enzymes that hydrolyze mannan (Yoon et al., 2010; Radfar et al., 2013). Daskiran et al. (2004) reported a positive effect of β-mannanase in broilers fed a soybean meal diet. Opoku et al. (2012) reported significant positive interactions between enzyme (protease and β-mannanase) and levels of wDDGS on AME and NR. However, there were no effects on performance of turkey poults (7 -21 d). Protease on the other hand is capable of degrading grain storage proteins and liberating higher levels of available amino acids (Barletta, 2012). Further research needs to be conducted to determine the optimum β-mannanase and/or protease source and/or levels in feed (Wu et al., 2005).

Livestock utilizes approximately 8% of the global water supply (Schlink et al., 2010). Water consumption is not regularly monitored or reported in animal trials (Viana et al., 2010), but is often referred to as an essential nutrient. Water intake is a factor of dietary ingredients and diet formulations (Shaw et al., 2006). This is important as excess water consumption will result in wet litter and lead to increased management and disease challenges. There is insufficient information with regards to the effects of feeding turkey hens' higher levels of wDDGS-based diets supplemented with either protease or β-mannanase on performance, including water intake.

4.3 Materials and methods

The use of animals in this trial was approved by the Animal Care Committee of the University of Saskatchewan (animal use protocol no. 19940248) and was performed in accordance with recommendations of the Canadian Council on Animal Care (1993) as specified in the Guide to the Care and Use of Experimental Animals.

4.3.1 Diets formulation and assay diets

The wDDGS was sourced from the Husky bioethanol plant (Lloydminster, Saskatchewan, Canada). There were two basal diets (0 or 30% wDDGS; Table 4.1) for the starter (0-28 d); grower (28-52 d); and finisher (52-72 d) phases, formulated to either meet or exceed the requirements of the Hybrid Converter turkey hen (http://www.hybridturkeys.com/hybrid- resources/nutritional-guidelines). These diets were formulated (Cargill Animal Nutrition, North Battleford, Saskatchewan) to be isonitrogenous and isocaloric. The diets were mixed in equal proportions at the University of Saskatchewan feed mixing facility to obtain a 15% wDDGS inclusion level. The highest percentage of wDDGS (30%) was then partitioned into 3, and 2 portions were supplemented with either protease (P+; 0.125g/kg) or β-mannanase (M+; 0.5g/kg). Enzymes were supplied by Jefo Nutrition Inc. (5020 Avenue Jefo, C.P. 325, St-Hyacinthe, Québec, Canada, J2S 7B8). The five diet treatments were 0% wDDGSE-, 15% wDDGSE- 30% wDDGSE-, 30% wDDGSP+ and 30% wDDGSM+.

Table 4.1. Composition of experimental diet (30%; with and/or without protease (0.125 g/kg) or β-mannanase (0.5 g/kg)) fed to turkey hens (0-72 d)

	Starter		Grower		Finisher	
	Level of wDDGS inclusion					
Item	0%	30%	0%	30%	0%	30%
Ingredient						
Wheat DDGS	0.00	30.00	0.00	30.00	0.00	30.00
Wheat - ground	45.70	15.10	35.45	0.00	63.00	0.00
Corn - ground	0.00	14.60	10.00	27.59	0.00	43.13
Soybean meal	20.50	15.60	14.77	24.66	0.00	5.25
Canola meal	9.32	0.00	10.00	0.00	7.98	0.00
Pork meal soya[a]	18.00	18.00	20.00	20.00	26.74	16.36
Fat	2.79	3.00	1.99	5.88	0.00	2.56
Dicalcium phosphate.	1.62	1.20	1.51	1.90	0.39	0.41
Trace mineral premix[b]	0.08	0.08	0.08	0.08	0.08	0.08
Calcium carbonate	0.81	1.23	2.01	3.93	0.00	0.85
Salt	0.24	0.29	0.17	0.09	0.16	0.07
Broiler vitamin premix[c]	0.06	0.06	0.06	0.06	0.06	0.06
Choline chloride (60%)	0.08	0.08	0.08	0.08	0.08	0.08
DL-Methionine	0.19	0.18	0.35	0.35	0.08	0.09
L-Lysine HCL	0.00	0.56	0.60	0.70	0.19	0.43
Vitamin D - HyD	0.07	0.07	0.07	0.07	0.07	0.07
Sodium Sequicarb	0.04	0.19	0.07	0.05	0.05	0.00
Celite	0.50	0.50	0.50	0.50	0.50	0.50
Calculated nutrient profile						
AME (kcal/kg)	2760	2760	2750	2750	2900	2900
Crude protein (%)	28.00	28.00	27.00	27.00	25.00	25.00
Fat (%)	6.00	8.43	5.50	8.94	4.11	7.15
Ash (%)	7.72	7.55	5.22	5.31	3.88	4.46
Calcium (%)	1.50	1.50	2.00	2.12	1.25	1.14
Phosphorus (%)	1.14	1.03	1.10	1.03	0.96	0.92
Lysine (%)	1.73	1.75	1.62	1.59	2.18	1.27
Methionine (%)	0.62	0.64	0.60	0.60	0.47	0.47
Threonine (%)	0.98	1.01	1.00	1.01	0.84	0.82
Met + Cys (%)	1.12	1.14	1.08	1.06	0.93	0.88

[a]ME, 3006 kcal/kg; Protein, 51.6%; fat, 10.9%; fiber, 3.86%; calcium, 4.40%; phosphorus, 2.42; potassium, 1.53; sodium, 0.30; arginine, 7.37%; histidine, 2.31%; isoleucine, 4.00; leucine, 7.42; lysine, 5.98; methionine, 1.52; phenylalanine, 4.33%; threonine, 3.78; tyrosine, 1.06; valine, 5.02%.

wDDGS=Wheat distillers dried grains with solubles

[b] Supplied per g or kg of diet: iron, 120 mg/kg; zinc, 117 mg/kg; manganese, 110 mg/kg; copper, 22 mg/kg; iodine, 1.5 mg/kg; selenium, 0.3 mg/kg

[c] Supplied per g or kg of diet: vitamin A (retinyl acetate + retinyl palmitate), 14.1 IU/g; vitamin D, 3.90 IU/g; vitamin E (dl-α-topheryl acetate), 42 IU/kg; thiamine, 3.0 mg/kg; riboflavin, 8.4mg/kg; niacin, 60mg/kg; vitamin $B_{6,}$ 6.0 mg/kg; vitamin K, 3.60mg/kg; vitamin B_{12}, 0.02 mg/kg; pantothenic acid,18 mg/kg; folic acid, 1.32mg/kg; biotin, 0.18 mg/kg

4.3.2 Experimental birds and management

A total of 700 0 d hybrid converter female turkeys (Charison's Hatchery, 89098 Road 7E, Gunton, MB, R0C 1H0) were randomly selected and weighed in groups of 35 (poult average weight was 55.0 ± 0.77 g). These groups of 35 poults were allocated to 20 floor pens measuring 3 m × 3 m in a completely randomized design. There were four replicate pens assigned to each of the five treatments from 0-72 d of age. Early brooding (0-7 d) conditions included supplemental heat lamps and brooder rings.

The birds were exposed to 23L:1D (L, light; D, dark) daily for the first week and then day length was decreased to18L:6D and maintained until the end of the trial. Light was provided by incandescent bulbs. Temperature was maintained at 30°C for the first 14 d and gradually stepped down to 22°C by 35d, individual heat lamps were used for the first 7 days in each pen. The temperature was then held at 19-22°C to 72 d. Feed and water were provided ad libitum throughout the trial. In the case of water, this was provided in 4 L temporary water founts during the first 7 d. After 7 d, poults were given access to the entire floor pen and bell-drinkers were provided. The bell drinkers in each pen were attached to a system that allowed monitoring of water consumption per pen; and this was done from 7 to 72 d (Figure 4.1).

Figure 4.1: Water measuring apparatus allows measurement of water intake from single bell drinkers.

4.3.3 Data collection

4.3.3.1 Growth performance

The measurement of average BW (kg) and FI (g/bird/d) was completed on a pen basis at 0, 28, 52, and 72 d. Feed conversion ratio (FCR; g feed:g gain) corrected for mortality was calculated. Post-mortem analysis on dead birds was carried out at the Prairie Diagnostic Services at the University of Saskatchewan to identify cause of mortality.

On the last day of the experiment (72 d), four birds in each replicate were humanely killed by cervical dislocation using a burdizzo. Similar intestinal segments measurements as described in chapter 3 (section 3.3.2.1) were recorded. Weight of abdominal fat pad was also

recorded. All measurements of the gut segments and abdominal fat were expressed relative (%) to the BW of the individual bird.

4.3.3.2 Ileal digesta collection and chemical analysis

For apparent ileal digestible energy (IDE) and NR, distal ileal (2 cm from the diverticulum) was gently squeezed using a roller vial to collect contents (same birds used for intestinal measurements). Samples were immediately frozen (-20C) and maintained at that temperature and then freeze dried. Following freeze-drying samples from birds in each replicate were ground using a mortar and pestle and pooled together. All analyses were done in duplicate. Refer to chapter 3 (section 3.3.3) for details on chemical analysis.

4.3.3.3 Water consumption

Figure 4.1 shows the water measuring system. A load-cell (capacity of 11 kg) holds a water reservoir (102 mm diameter x 330 mm length plastic drain pipe); and a solenoid valve controls the flow of water in and out of the reservoir. Based on the changes in weight as the reservoir empties and fills, the amount of water consumed is monitored.

4.3.3.4. Litter moisture

Litter samples for moisture determination were taken on 52 and 72 d of the experiment. Four locations away from drinker and feeder were marked in each of the 20 pens for sampling. These locations differed from pen to pen due to inconsistent positioning of feeders and drinkers. Four samples (2 x 2 cm) of litter were taken from each pen and weighed. The samples were placed in a drying oven (55^{o}C) until weights were stable. Change in weight during drying was expressed as percentage (%) moisture of litter.

4.3.4 Calculations

Formulas used for calculation of apparent IDE and NR are from those by Scott and Hall (1998) on digestibility calculation.

Apparent IDE (kcal/kg of diet) = $GE_{diet} - [GE_{digesta} \times (Marker_{diet}/Marker_{digesta})]$

$NR = 100 - [100 \times (\%\ Maker_{diet}/\%Marker_{digesta}) \times (\%\ N_{digesta}/N_{diet})]$

4.3.5 Statistical analysis

Data was analyzed as a completely randomized design using Proc GLM (General Linear Model) of SAS version 9.2 (SAS Institute Inc, 1996). The experimental unit was a pen of 35 birds. Data were considered significant when $P \leq 0.05$. Duncan's Multiple Range Test was used to separate significant mean values.

4.4 Results

The composition of wDDGS used in the diet formulation was 93.3% DM, 35.9% CP, 4.57% fat and 7.22% crude fiber. The analyzed dry matter and crude protein of the diets are presented in Table 4.2.

Table 4.2. Analyzed nutrient composition of dietary treatment (as-fed)

	Starter (0-28 d)					Grower (28-52 d)					Finisher (52-72 d)				
	Level of wDDGS inclusion														
Item	0%	15%	30%	30%P+	30% M+	%0	15%	30%	30% P+	30% M+	%0	15%	30%	30% P+	30% M+
Dry matter	91.4	91.6	92.2	92.5	92.4	90.6	91.7	91.5	92.0	91.9	89.4	89.9	89.9	89.8	90.0
Crude protein	26.8	28.1	28.9	29.4	29.3	27.5	27.3	27.8	27.3	27.8	25.7	25.0	25.6	24.7	24.7

P+ = Protease ((P+; 0.125g/kg)

M+ = β-mannanase (M+; 0.5g/kg)

wDDGS=Wheat distillers dried grains with solubles

4.4.1 Growth performance

Average performance for all treatments is presented in Table 4.3. There were no treatment effects on 28 and 72 d BW. At 52 d of age the birds fed 30% wDDGSP+ were significantly heavier than birds fed without enzyme and containing either 0 or 15% wDGGS. The weight of poults fed the 30% wDDGSP+ diets were not different from those fed the 30% wDDGS diets with or without β-mannanase; and poults from the latter two diets were not different from those fed 0 or 15% wDDGS without enzymes. There was no difference in FI (g/bird/d) between the five dietary treatments. The results also indicated no differences in FCR from 0-28 and 52-72 d. The FCR values for 28-52 and 0-72 d were different.

During the 28-52 d (grower phase), the 0% wDDGSE- treatment resulted in a higher FCR than the other four diets that were not different from each other. In this same phase, the 30% wDDGS diet with either P+ or M+ had lower FCR, but these were not different from 15% wDDGSE-. Similarly, the FCR for 0-72 d (total) was higher for 0% wDDGSE-; whereas the other four dietary treatments showed no differences. No treatment difference was noted for mortality (%) in any of the periods. Highest mortality (% total) was reported for wDDGSP+ (5.6%) and lowest for wDDGSM+ (2.1%). Poults fed 30% wDDGSP+ had a higher incidence of pendulous crop (10.4%; included culled birds for mortality and those that were noticed on the last day of experiment before transporting) than birds from the other treatments.

Table 4.3. Effects of wheat distillers dried grains with solubles (0, 15; without enzymes or 30%; with and/or without protease (0.125 g/kg) or β-mannanase (0.5 g/kg)) on average body weight, feed consumption, FCR, mortality and % pendulous crops of turkey hens (0-72 d)

	Level of wDDGS inclusion						
Item	0%	15%	30%	30%P+	30%M+	SEM	P-Value
Average body weight (kg/b)							
28 d	0.968	0.955	0.980	1.000	1.000	0.018	NS
52 d	3.43^{c}	3.49^{bc}	3.60^{ab}	3.68^{a}	3.63^{ab}	0.049	*
72 d	6.39	6.40	6.40	6.59	6.47	0.065	NS
Feed intake (g/b/d)							
0-28 d	46.5	45.1	48.4	46.8	46.2	1.02	NS
28-52 d	189	186	191	190	188	2.7	NS
52-72 d	379	369	362	370	364	6.9	NS
0-72 d	186	182	182	183	181	2.7	NS
FCR (g feed:g gain)							
0-28 d	1.43	1.41	1.47	1.39	1.38	0.025	NS
28-52 d	1.85^{a}	1.77^{b}	1.75^{b}	1.71^{b}	1.72^{b}	0.021	**
52-72 d	2.56	2.54	2.58	2.55	2.56	0.034	NS
0-72 d	2.12^{a}	2.07^{b}	2.08^{ab}	2.03^{b}	2.04^{b}	0.018	*
Mortality (%)							
0-28 d	4.17	2.80	2.78	3.50	2.09	0.566	NS
28-52 d	0.70	0.00	1.39	0.00	0.00	0.695	NS
52-72 d	0.00	2.08	0.00	2.09	0.00	1.105	NS
0-72 d	4.87	4.88	4.17	5.58	2.09	1.300	NS
Pendulous crops (%)	2.10^{b}	2.78^{b}	4.88^{b}	10.38^{a}	4.86^{b}	1.486	*

SEM=Standard error of means

Means with different superscripts within the same row are significantly different *$P \leq 0.05$; **$P \leq 0.01$

P+ = Protease ((P+; 0.125g/kg)

M+ = β-mannanase (M+; 0.5g/kg)

wDDGS=Wheat distillers dried grains with solubles

4.4.2 Water intake

The results on water consumption are summarized in Table 4.4. Average water consumption showed a tendency (P=0.08) to be higher for the 30% wDDGSP+ treatment from 7-28 d. Higher water consumption from 28-52 d was found for 30% wDDGSP+; but the other four dietary treatments (0%, 15%, 30% and 30%M+) were not statistically different. Overall water consumption (7-72 d) tended to be higher (P=0.07) for 30% wDDGSP+. No difference was recorded on water:gain ratio between the periods of 28-52 d and 28-72 d (the water:feed ratio could not be calculated for 0-28 d because water intake was not measured from 0-7 d, only from 7-28 d). A difference in water:gain ratio was observed for the finisher phase (52-72d). This ratio was greater for 30% wDDGSE- and 30% wDDGSP+; whereas 0% wDDGSE-, 15% wDDGSE- and 30% wDDGSM+ were not different from each other. There was no differences in water:feed ratio between dietary treatments for all growth phases. Nonetheless, birds fed the 30% wDDGSP+ diet between 28-72 d recorded a numerically (2.38; P=0.15) higher water:feed ratio, whereas those fed diets containing 15% wDDGSE- recorded the lowest water:feed ratio (2.20).

The data on litter moisture is not shown as there were no differences observed between dietary treatments. The overall litter moisture recorded at 52 and 72 d were 30.2 and 30.1%, respectively.

Table 4.4. Effects of wheat distillers dried grains with solubles (0, 15; without enzymes or 30%; with and/or without protease (0.125 g/kg) or β-mannanase (0.5 g/kg)) on average water consumption (mL/bird/d), water:gain and water:feed of turkey hens (7-72 d).

	Level of wDDGS inclusion						
Item	0%	15%	30%	30%P+	30%M+	SEM	P-Value
Water consumption (mL/b/d)							
7-28 d	172	168	174	202	177	8.5	P=0.08
28-52 d	419^{b}	411^{b}	418^{b}	453^{a}	423^{b}	8.0	*
52-72 d	610	581	612	634	602	14.1	NS
7-72 d	356	330	342	360	341	9.9	P=0.07
Water:gain (L:kg)							
28-52 d	4.09	3.89	3.83	4.05	3.86	0.086	NS
52-72 d	4.11^{b}	4.01^{b}	4.37^{a}	4.36^{a}	4.24^{ab}	0.075	*
28-72 d	4.10	3.95	4.10	4.21	4.05	0.068	NS
Water:feed (L:kg)							
28-52 d	2.21	2.20	2.19	2.38	2.25	0.054	NS
52-72 d	1.61	1.58	1.69	1.71	1.66	0.039	NS
28-72 d	1.84	1.81	1.89	1.97	1.88	0.043	NS

SEM=Standard error of means
Means with different superscripts within row are significantly different *P≤0.05
P+ = Protease ((P+; 0.125g/kg)
M+ = β-mannanase (M+; 0.5g/kg)
wDDGS=Wheat distillers dried grains with solubles

4.4.3 Relative gut measurement

There was no impact of dietary treatment on any of the intestinal segment measurements (Table 4.5) except for relative weight of crop and relative length of caeca. For relative crop weight, the 30% wDDGSE- or M+ values were heavier than the crops from birds fed the 15% wDDGSE- diet; the 0% wDDGSE- and 30% wDGGSP+ diets were intermediate and not different from the above diets. Relative caecal length was longer for birds from the 30% wDDGSM+ and 30% wDDGSE- treatments as compared to poultry from 0% wDDGSE- and 30% wDDGSP+; the 15% wDDGSE- diet was intermediate and not different from the above.

Table 4.5. The effect of different inclusion levels of wheat distillers dried grains with solubles (0, 15; without enzymes or 30%; with and/or without protease (0.125 g/kg) or β-mannanase (0.5 g/kg)) on empty gastrointestinal segments and abdominal fat pad of turkey hen (0d-72d)

	Level of wDDGS inclusion						
Item	0%	15%	30%	30%P+	30%M+	SEM	P-Value
Proventriculus weight (%)	0.175	0.179	0.171	0.174	0.182	0.005	NS
Crop weight (%)	0.217^{ab}	0.206^{b}	0.256^{a}	0.223^{ab}	0.248^{a}	0.013	*
Gizzard weight (%)	1.33	1.32	1.25	1.30	1.38	0.037	NS
Duodenal weight (%)	0.235	0.245	0.231	0.232	0.229	0.001	NS
Jejunum weight (%)	0.700	0.674	0.653	0.646	0.619	0.024	NS
Ileal weight (%)	0.482	0.513	0.489	0.473	0.492	0.016	NS
Duodenal length (%)	0.619	0.623	0.623	0.611	0.592	0.015	NS
Jejunum length (%)	1.42	1.44	1.40	1.38	1.45	0.038	NS
Ileal length (%)	1.42	1.41	1.36	1.40	1.41	0.037	NS
Caeca length (%)	0.885^{c}	0.894^{bc}	0.977^{ab}	0.886^{c}	1.01^{a}	0.030	**
Abdominal fat pad (%)	1.00	1.08	1.22	1.17	1.20	0.078	NS

SEM=Standard error of means. Means with different superscripts within row are significantly different *P≤0.05; **P≤0.01
NS= non-significant
P+ = Protease (P+; 0.125g/kg)
M+ = β-mannanase (M+; 0.5g/kg)
wDDGS=Wheat distillers dried grains with solubles

4.4.4 Digestibility

There was a higher apparent IDE for 30% wDDGSP+ but was not different from 30% wDDGSM+ or 15% wDDGSE- (Table 4.6). The apparent IDE was significantly lower for 0% wDDGSE- but not statistically different from 15% wDDGSE- or 30% wDDGS with or without β-mannanase. Similarly, a higher NR was reported for 30% wDDGSP+. There was no difference between 0% wDDGSE-, 15% wDDGSE- and 30% wDDGSM+. A lower NR was recorded for 30% wDDGS but not statistically different from 30% wDDGSM+.

Table 4.6. Effects of wheat distillers dried grains with soluble (0, 15; without enzymes or 30%; with and/or without protease (0.125 g/kg) or β-mannanase (0.5 g/kg)) on apparent ileal digestible energy (IDE) and nitrogen retention of turkey hens at 72 d of age

	Level of wDDGS inclusion						
Item	0%	15%	30%	30%P+	30%M+	SEM	P-Value
IDE (kcal/kg)	2607^{b}	2844^{ab}	2801^{b}	3179^{a}	2871^{ab}	61.0	*
Nitrogen retention (%)	75.8^{b}	76.7^{b}	70.0^{c}	81.9^{a}	72.5^{bc}	1.12	**

SEM=Standard error of means
Means with different superscripts within the same row are significantly different *$P \leq 0.05$; **$P \leq 0.01$
P+ = Protease (P+; 0.125g/kg)
M+ = β-mannanase (M+; 0.5g/kg)
wDDGS=Wheat distillers dried grains with solubles

4.5 Discussion

The use of DDGS in poultry diets has increased significantly as more information on nutrient availability and how processing impacts its nutritional value is identified. Our focus is on wDDGS, and adds to the limited information on wDDGS utilization by turkeys (Roberson, 2003; Noll and Brannon, 2006; Opoku et al., 2012). The current experiment evaluated levels of wDDGS on birds' performance and water consumption. Furthermore, at the highest level of wDDGS (30%) the use of supplemental protease or β-mannanase were tested.

The health and performance (i. e., in terms of BW) of the turkeys in this trial was acceptable (compared to the hybrid goals), although average FCR was approximately 15% higher as compared to the FCR provided in the standards for Hybrid Converter turkeys (http://www.hybridturkeys.com/hybrid- resources/nutritional-guidelines). However, BW was higher for all dietary treatments compared to the standards (~10%). This is a reflection of the higher FI of birds. Birds consumed approximately 24.6% more feed compared to what was expected relative to the Hybrid guideline. Diets were mash which is expected to reduce FI as compared to pelleted diets (Briggs et al., 1999; Scott, 2002). The results obtained in this experiment are similar to those obtained by Olukosi et al. (2010). Wang et al. (2007) fed corn DDGS (0, 15 and 30%) to broilers and reported no negative effect on growth performance at 15% inclusion but feeding 30% depressed performance. This was attributed to the amino acid deficiency (arginine) in the diet containing 30% DDGS. Cozzanet et al. (2010) reported that lysine and arginine are variable (1.7-3.0% for lysine and 3.7-4.6% for arginine) in DDGS; probably due to excessive heating causing Maillard reactions (Bolarinwa and Adeola, 2012; Noblet et al., 2012). Noblet et al. (2012) has also reported about 3.1 to 3.3% for lysine nonheat-damaged corn DDGS compared to 2.10% in heat-damaged corn DDGS. There were no measurements of available (reactive) lysine in these experiments, hence the influence of processing on available lysine in diets is not known. In the current experiment, feeding diets containing 30% wDDGS did not depress performance compared to the control diet; and a significant correlation ($P<0.01$; $R^2= 0.60$; data not shown) was found between FI and BW.

A number of factors vary in the production of DDGS, these include the removal of the oil-rich germ and fiber-rich hulls preceding fermentation to improve ethanol yield (now practiced increasingly in corn used for ethanol production) and/or removal of oil from the thin stillage;

fermentation and the drying processes; as well as the differences in addition of solubles (Spiehs et al., 2002; Bregendahl, 2008). Soluble non-starch polysaccharide (NSP) is high in wheat; however, this material is hydrolyzed during fermentation and typically not a factor with respect to formulation and requirements for NSPase. Depending on the subsequent heat treatments there may be more insoluble NSP that becomes soluble. Therefore, higher inclusion of DDGS could cause a decrease in nutrient and energy availability. The lack of differences in the performance of birds fed the 30% wDDGS with or without enzymes (protease or β-mannanase); and an improvement in the performance of turkey hens fed the higher levels of wDDGS compared to the control diet was unexpected. This may be a consequence of the improvements in processing of DDGS and a resulting increase in nutrient availability (Waldroup et al., 2007). Additionally, nutrient availability may relate to differences in solubles inclusion (Waldroup et al., 2007). This may also be associated with variability in yeast cell wall residuals which can account for up to 5% of the total protein in DDGS (Noblet et al., 2012). It is apparent from this study that, the 30% inclusion of wDDGS when adequately supplemented with energy and amino acids was not detrimental to overall performance. Wheat on the other hand, contains 5-8% NSP pentosans; hence when included at higher levels (as was the case of the 0% wDDGS as compared to 15 or 30% wDDGS diets in this study) in poultry diets, it may reduce nutrient digestibility and performance (Carre and Brillouet, 1986; Choct and Kocher, 2000). However, as indicated earlier, overall performance for all diets was superior to that provided by the Hybrid guidelines.

The levels of mannan associated with yeast cell wall residue may result in reduced nutrient availability. Patel and McGinnis (1985) reported a significant negative effect of mannan on layer performance. Daskiran et al. (2003) showed a significant improvement in the overall feed utilization when β-mannanase was supplemented in corn-soybean diet containing guar gum

(i.e., high in mannan). The increased performance was attributed to the significantly improved metabolizable energy and reduced total nitrogen excreted with β-mannanase supplementation. Similarly, Jackson et al. (1999) reported an increase in egg weight during early production and increased egg numbers with diets supplemented with β-manannase. This signifies that, diets with higher mannan content respond to β-manannase. In the present study, β-manannase in 30% wDDGS based diets was not effective; supporting an earlier study with young turkey poults (Opoku et al., 2012).

Adeola and Cowieson (2011) reported promising effects for protease in the diets of swine and poultry. This is contrary to the results of the present experiment. Adeola and Cowieson (2011) speculated that protease enzymes may have positive or negative interactions with exogenous and endogenous enzymes and that, further research is required to clarify this. Zhou et al. (2009) reported a higher response to enzyme supplementation (e.g. protease, xylanase, and amylase) when diets were formulated to be lower in nutrients. The energetic effect of an enzyme is increased in diets with low added fat (Zanella et al., 1999; Adeola and Cowieson, 2011). Enzyme supplementation can improve the absorption of fats and oils by reducing viscosity of digesta. In the case of this experiment, the numerical increase in relative abdominal fat weight at higher wDDGS level indicates that the energy levels were likely higher due to the extra supplemental fat used to balance the diets. Diets were not isocaloric as we found significant differences in apparent IDE; it was highest for 30% wDDGSP+. Supplemental fat increased abdominal fat deposition (Griffiths et al., 1977; Deaton and Lott, 1984). Crespo and Garcia (2001) reported a constant abdominal fat when sunflower oil was fed to broilers as compared to an increase abdominal fat pad deposition when tallow was added to the diets. These authors

concluded that depending on the fatty acid profile, increased dietary fat resulted in higher metabolizable energy may not necessarily result in abdominal fat deposition.

Protease addition to diets with 30% wDDGS caused a numerical increase in 52 d BW (1.4% and 2.2% as compared to 30% wDDGS and 30% wDDGSM+, respectively). The 52 d BW of turkeys fed the 30% wDDGSP+ was higher than for birds fed 0% wDDGS (~6.8%) or 15% wDDGS (~5.2%). Consistent with the findings in the current experiment, Yu et al. (2007) reported a numerical increase in BW and a significantly lower FCR when broilers were fed a corn-soybean diet supplemented with protease. Thacker (2005) also reported a significant improvement in FCR when protease was supplemented in a wheat-based diet. These authors recorded no improvement in either total tract digestibility of protein or energy digestibility in the respective studies. Although protease can improve digestibility of dietary protein or amino acids (Isaksen et al., 2012), the studies by Thacker (2005) and Yu et al. (2007) suggest that there could be other factors besides protease's effect on protein degradation. This calls for further investigation. Ghazi et al. (2002) showed an improvement in the nutritive value of soybean meal when supplemented with an acid fungal protease rather than an alkaline subtilisin. Proteases are often fed in combination with other enzymes and the additive effects of different enzyme activities needs further investigation. Higher BW of turkeys fed 30% wDDGSP+ may have also been related to birds with larger crops, although birds were culled for pendulous crops (i.e., crop prolapse), those with less severe problems may have been missed. The higher weight of the material in developing pendulous crops may be partly responsible for this observation.

The current experiment is one of few studies to compare the impact of diet on water intake, and the first to our knowledge with respect to inclusion of wDDGS. There were trends for higher water intake (expressed as litres/bird or litres:kg feed) with 30% wDDGSP+ diets as

compared to the other diets. This diet was also associated with higher incidence of pendulous crop and may explain the higher water intake; however the reason for higher pendulous crop is still uncertain. Pendulous crop in poultry is characterized by temporary or permanent distension of the crop with a stagnant liquid or semi-liquid content (Wheeler et al., 1960). According to Wheeler et al. (1960) this condition may lead to death of birds due to rupture of the crop or from under nourishment (as in most cases birds refuse to eat), or as a consequence of improper feed digestion and assimilation. Although overall mortality in the current study was low, significant treatment effects were found for pendulous crops. The principal cause of mortality was due to culled birds for pendulous crop (i.e., to minimize condemnations at processing); and was significantly higher for 30% wDDGSP+ diets as compared to the other four diets. Wheeler et al. (1960) further noted that the amount of crude protein (CP) present in the diets could trigger the occurrence of pendulous crop. The analyzed protein content of the dietary treatments was similar, with the exception of the starter phase that indicated increasing CP with increasing wDDGS inclusion (Table 4.3). At 72 d the NR was significantly higher for 30% wDDGSP+. It may also be a factor of protease itself, causing a weakening of the crop and hence higher incidence of prolapse.

The relative caecal length was highest for birds fed 30% wDDGS with β-mannanase enzyme, but was significantly less for 30% wDDGSP+. The caeca acts as the primary site for microbial fermentation of complex carbohydrates such as fiber, which resist degradation in the lower digestive tract (Remington, 1989; Klasing, 1998; Svihus et al., 2013). The fermentation by microflora results in the production of volatile fatty acids (Svihus et al., 2013). Further work is required to understand if this interaction between protease supplementation and changes in the intestinal tract segment are due to hydrolysis of protein, damage to segment structure or a factor

of higher water intake. Generally, all diets with 30% wDDGS, regardless of enzyme supplementation recorded higher relative crop weight and could be explained by a higher level of fiber.

It is critical to recognize that the requirements of water for the same species of animal may differ (Schlink et al., 2010) due to differences in diet, feed form, temperature, BW, housing and environmental stress (Patience et al., 2005, Shaw et al., 2006; Eichner et al.,2007; Schlink et al., 2010). Shaw et al. (2006) reported an increase in the amount of urine excreted by pigs fed a high protein diet compared to a low protein diet, resulting from excreting of excessive nitrogen produced during metabolism of excess protein for energy.

The higher consumption of water with birds fed 30% wDDGSP+ could have resulted in higher litter moisture. However, litter moisture (i.e., approximately 30%) was low and was not different between treatments. Similar results were reported by Macklin et al. (2005) and Eichner et al. (2007). This may indicate that the ventilation was effective in removing differences. To date there is no scientific literature on the effects of wDDGS and enzyme supplementation on water consumption in turkey hens. Hence, it is difficult to understand the association between protease supplementation and water intake.

With respect to water:gain ratio, Daskiran et al. (2003) showed a decreased water:gain ratio in broilers when a corn-soybean diet was supplemented with β-mannanase. This is similar to what was recorded in the current experiment when β-manannase was supplemented. Even though water:gain ratio for 30 wDDGSM+ was not significantly different from either 30% wDDGS with/without P+; supplementation of M+ reduced water intake by 1.3 and 3.0% as compared to 30% wDDGS and 30% wDDGSP+, respectively. As cited by Daskiran et al. (2003), Read (1986) reported that a reduction in gastric emptying rate, disrupts the mixing of

substrate with digestive enzymes, and decreases contact of nutrients with the absorptive epithelium. Therefore, birds increase their level of water consumption to maintain proper mixing of digestive enzymes with substrates and this result in higher litter moisture.

This experiment is the first to use the water intake apparatus (Figure 4.1) at the Poultry Research Centre. The large amount of data produced is difficult to tabulate and may have resulted in some lack in precision in measuring water intake. Similarly, there may have been differences in water wastage, although this was not shown in assessment of litter moisture.

In conclusion, the results show no negative effects on performance of feeding higher levels of wDDGS (30%) to turkey hens. High levels of wDDGS with no loss of production would result in a higher demand for using wDDGS in turkey diets. However, the nutritional composition of wDDGS within and between ethanol plants can differ; hence regular chemical analysis is required to account for potential variability. This research has indicated that neither the source nor level of protease or β-mannanase tested were useful in improving the nutrient availability of 30% wDDGS.

Dietary composition could alter the water consumption pattern of birds. The present research has demonstrated an increase in water intake for turkeys fed high wDDGS diet supplemented with protease enzyme. The underlying reason for this effect on water intake is uncertain. Detailed research is required to understand this and to further determine water requirements for different poultry species.

5.0 THE EFFECTS OF EXTRUSION OF WHEAT DISTILLERS DRIED GRAINS WITH SOLUBLES WITH AND/OR WITHOUT AN ENZYME COCKTAIL ON PERFORMANCE TURKEY HEN POULTS

5.1 Abstract

Two experiments were conducted to determine if extrusion (EX) and/or enzymes (E) could overcome the restrictions (e.g., high fiber) of feeding wheat distillers dried grain with solubles (wDDGS) and improve its nutritional value for feeding turkeys. Two starter diets with either 0 or 30% wDDGS were formulated to meet or exceed the nutrient requirements of the Hybrid Converter female turkeys. The 30% wDDGS diet was either non-extruded (EX-) or extruded (EX+) wDDGS. Three basal diets were then produced [0% wDDGS (EX-) or 30% wDDGS (EX-/EX+)]. Diets were blended to obtain 15% wDDGS. In the respective treatments, only wDDGS was extruded (temperature; 118°C, retention; 1min 55 sec, total moisture; 25% and pressure 33 bar). The respective experimental diets were supplemented with/without an enzyme cocktail (E; 0.5g/kg). Test diets were fed from 7-21 d in a completely randomized design. In experiment 1, a total of 210 turkey hen poults were fed diets containing 0, 15 or 30% wDDGS (EX-) with or without enzyme (E+/E-). Body weight and FI were significantly higher for 0% wDDGSE-. The NR and AME for the 30% wDDGSE- was significantly higher than other treatments at 21 d. The results indicated significant main effects of E and an interaction between wDDGS level and E. In experiment 2, 280 turkey hen poults were fed eight diets [15/30% wDDGS (E+/E-), (EX-/EX+)]. The level of wDDGS had a significant effect on BW, FI and FCR; 15% inclusion was superior to 30%. There were significant 2-way and 3-way interactions for AME and NR at 21 d due to differences in enzyme response with 15 or 30% wDDGS inclusion and/or extrusion of wDDGS. As high as 15% wDDGS can be incorporated in turkey hen diets. There was no beneficial effects of EX or E on poults performance.

5.2 Introduction

The production of renewable energy as ethanol from cereal grains has increased over the years to supplement fuel requirements (Thacker and Widyaratne, 2007; Shalash et al., 2009). Wheat serves as the major substrate for ethanol production in Western Canada and some parts of Europe (Avelar et al., 2010). Ethanol production via wheat has accounted for the production of ~1.4 MMT of wheat distillers dried grains with solubles (wDDGS) from the biofuel industry (Ethanol Producer Magazine, 2013). This is available for use as a feed ingredient in poultry (Noll et al., 2001; Avelar et al., 2010; Dozier, 2012; Leeson et al., 2012). During ethanol production the ground cereal is converted into simple sugars via enzymatic action followed by fermentation with yeast to produce ethanol and the co-product DDGS and CO_2 (Rosentrater 2006; Fallahi et al., 2013). There are limits in inclusion of DDGS in monogastric diets due to high fiber and amino acids (especially low lysine) (Lee et al., 2003; Lumpkins et al., 2004; Lim et al 2009; Fallahi et al., 2013). There are also concerns about low energy due to the conversion of starch to ethanol (Chevanan et al., 2008; Kerr and Shurshon, 2013) and the removal of fat from modern ethanol production (Wisner et al., 2013). Emerging technologies (e.g., feed processes and/or enzymes) may be useful to ensure effective utilization of the nutrients tightly bound to this high fiber and high protein co-product (Fallahi et al., 2013; Kerr and Shurshon, 2013).

An enzyme cocktail or a multi-enzyme complex could more effectively degrade complex matrixes of fibrous carbohydrates or indigestible cell wall components of feed ingredients (Cowieson and Adeola, 2005; Emiola et al., 2009; Adeola and Cowieson, 2011; Kalmendal and Tauson, 2012; Kerr and Shurshon, 2013). This will reduce their antinutritive effect, enhance digestion of nutrients and subsequently improve performance in diets of monogastric animals (Cromwell et al., 1993; Emiola et al., 2009; de Vries et al., 2012; Ziemer et al., 2012).

Extrusion has found application in aquaculture (Chevanan et al., 2009, 2010; Ayadi et al., 2011; Fallahi et al., 2013), human (Hood-Niefer and Tyler, 2010), pet (Muthukumarappan, 2012) diets and to a limited extent in poultry and swine feeds (Fadel et al., 1988; Vukic-Vranjes et al. 1994; Vukic-Vranjes and Wenk 1995; Gracia et al., 2003; Oryschack et al., 2010a, b; Opoku et al., 2013). Extrusion is a hydrothermal process that uses combinations of temperature, moisture, pressure, shear, and mixing with variable time to modify the physical and nutrient structure of diets and/or ingredients (Fallahi et al., 2013). Ayadi et al. (2011) summarized advantages of extrusion, including: reduced antinutritional factors; improved palatability; and better digestibility. Oryschak et al. (2010a) reported that single screw extrusion of triticale DDGS significantly improved amino acid digestibility in poultry.

This study will investigate if extrusion and/or supplementation with an enzyme cocktail will positively impact the utilization of wDDGS diets by turkey hen poults.

5.3 Materials and methods

All procedures involving animal handling and testing were reviewed and approved by the University of Saskatchewan Committee on Animal Care and Supply (animal use protocol no. 19940248) and followed the principles established by the Canadian Council on Animal Care (1993).

5.3.1 Test Ingredients and extrusion process

The wDDGS used in the current experiment was a product from Husky ethanol processing plant (Lloydminster, Saskatchewan, Canada). The wDDGS used in the diets were either non-extruded (EX-) or extruded (EX+). The wDDGS was extruded using a twin-screw extruder (Clextral Evolum 32, Firminy, France) with a 4.88 mm diameter die at the Saskatchewan Food Industry Development Centre Inc (Food Centre; 117-54 Innovation Boulevard Saskatoon, SK S7N 2V3, Canada). The extruder was powered by a 47.2-kW motor

with a maximum screw speed of 496 rpm, a torque of 11 Nm and a pressure of 33 bar. There were 6 barrel zones with varying temperatures of 29, 80, 101, 119, 118, and 118°C, respectively. The wDDGS with moisture 11.15% (as in) was extruded at a feeding rate of 54 kg/hr. The total residence time for extrusion was 1 minute 55 seconds. Extrudates with a total moisture of 25% then pass through a dryer (115°C for 2 min 25 sec) for drying and cooling. The extrudates produced lacked an expected nugget form due to previous denaturing of the protein during ethanol production and drying of wDDGS.

5.3.2 Particle size analysis

To ensure a standard particle size, the extruded and non-extruded wDDGS were ground using a hammer mill (Glen mills Inc, 203 Brookdale St. NJ; University of Saskatchewan, College of Engineering) with a 4.76 mm screen size. Particle size analysis was accomplished using a rotary-tap testing sieve shaker (Tyler Industrial Product, OH, USA). Four replications per each wDDGS (weight; 300 g) source were determined. After 10 minutes, material left on each sieve were weighed and recorded. The sieve mesh sizes used were US standard (12, 20, 30 50, 60 and 100) representing 1680, 841, 594, 297, 250 and 150 microns, respectively. Mean particle size (Dgw) and standard deviation (Sgw) was determined for each sample (ASAE, 2012).

5.3.3 Diets formulation and assay diets

Two diets containing 0% wDDGS or 30% wDDGS were formulated to either meet or exceed the nutrient requirements of Hybrid Converter turkey starter diet (http://www.hybridturkeys.com/hybrid-resources/nutritional-guidelines). Diets were formulated based on digestible amino acids (Oryschak et al., 2010). The 30% wDDGS diet contained either extruded (EX+) or non-extruded (EX-) wDDGS to produce 3 basal diets [0% wDDGS (EX-),

30% wDDGS (EX+) and 30% wDDGS (EX-) (Table 5.1). Only wDDGS was extruded in the respective mash diets. The diets were formulated to provide 27.5% CP, 2850 kcal of AME/kg, 1.62% lysine, 0.65% methionine, 1.40% calcium and 0.75% available phosphorus.

Table 5.1. Composition of experimental diets formulated to determine the effects of extrusion of wheat DDGS (wDDGS) with and/or without an enzyme cocktail (0.5 g/kg) on turkey hen poults performance

	0% wDDGS	30% wDDGS	
Ingredients		Extruded	Non-extruded
Wheat	47.19	37.83	37.83
Wheat DDGS	0.00	30.00	30.00
Soybean meal	36.43	14.03	14.03
Fish meal	2.00	2.85	2.85
Corn-gluten meal	4.93	4.21	4.21
Canola oil	3.29	4.79	4.79
Dicalcium phosphate	1.68	1.00	1.00
Limestone	2.72	3.01	3.01
Salt	0.28	0.30	0.30
Vitamin and mineral premix[a]	0.50	0.50	0.50
Choline chloride	0.07	0.07	0.07
DL-Methionine	0.17	0.17	0.17
L-Lysine	0.18	0.68	0.68
Celite	0.50	0.50	0.50
Calculated nutrient levels			
M.E (kcal/kg)	2850	2850	2850
Protein (%)	27.50	27.50	27.50
Fat (%)	5.11	8.33	8.33
Fiber (%)	2.65	4.04	4.04
Calcium (%)	1.40	1.40	1.40
Phosphorus (%)	0.75	0.75	0.75
Sodium (%)	0.17	0.17	0.17
Methionine (%)	0.65	0.65	0.65
Lysine (%)	1.62	1.62	1.62
Threonine (%)	1.04	0.94	0.94

[a]Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11000 IU; vitamin d, 2200 IU; vitamin E (dl-α-topheryl acetate), 30 IU; menadione, 2 mg; thiamine, 1.5 mg; riboflavin, 6 mg; niacin, 60 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; and biotin, 0.15 mg. iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper,10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.

5.3.3.1 Experiment 1

In experiment 1, two of the basal diets [(0% wDDGS (EX-) and 30% wDDGS (EX-)] were equally mixed to obtain 15% wDDGS (EX-) inclusion. These diets (0% wDDGS; EX- and 15% wDDGS; EX-, 30% wDDGS; EX-) were either supplemented with/without an enzyme cocktail [(Superzyme (E+); 0.5 g/kg, (Canadian Bio-System, 4389 112 Ave SE, Calgary, AB, T2C 0J7, Canada)]. The enzyme supplied 1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase, 7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase. The six assay test diets in experiment 1 were 0% wDDGS (E-), 0% wDDGS (E+), 15% wDDGS (E-), 15% wDDGS (E+), 30% wDDGS (E-) and 30% wDDGS (E+).

5.3.3.2 Experiment 2

In experiment 2, the 0% wDDGS (EX-) and the 30% (EX-, EX+), were mixed into equal proportions to obtain 15% wDDGS (EX-, EX+). The 15 wDDGS (EX-, EX+), 30% (EX-, EX+) were then supplemented with/without an enzyme cocktail (similar to experiment 1). The eight assay diets fed in experiment 2 were 15 wDDGS (EX-/E-), 15 wDDGS (EX-/E+), 15 wDDGS (EX+/E-), 15 wDDGS (EX+/E+), 30 wDDGS (EX-/E-), 30 wDDGS (EX-/E+), 30 wDDGS (EX+/E-) and 30 wDDGS (EX+/E+).

5.3.4 Experimental birds and management

A total of 490 one-day old Hybrid Converter turkey hens (Lilydale Hatchery, 7503 127 Avenue NW Edmonton, AB T5C 1R9, Canada) were placed in battery cages at the University of Saskatchewan Poultry Centre. For the first 7 d, birds were kept in groups of 10 and had free access to a standard wheat soybean turkey starter crumbled diet (Co-op Feeds, Saskatoon) providing 34.6% CP, 3020 kcal/kg of AME, 0.66% methionine, 1.73% lysine, 1.49% calcium

and 0.76% available phosphorus. On d 7, individually weighed turkey poults were wing banded and allocated into two experimental groups. Turkey poults were provided free access to feed and water. A standard brooding temperature starting from 32°C from 0 d and gradually reduced to 23°C at 21 d was used. The birds were exposed to 18L:6D (L:light, D:dark), with a light intensity of 10-20 lux.

5.3.4.1 Experiment 1

In experiment 1, 210 7 d old turkey poults were randomly assigned to 42 battery cages measuring 29.2 cm (height) × 48.3 cm (depth) × 83.8 cm (width; providing 1010 cm^2/bird at 21 d) in a completely randomized design. Poults were assigned to six different dietary treatments (described in experiment 1 above), with a total of five birds per cage and seven replicates per treatment.

On d 7, 14 and 21; BW and FI were recorded. Feed conversion ratio corrected for mortality was calculated. AME and NR determination and intestinal segments measurements were as described in chapter 3 (section 3.3.2.1).

5.3.4.2 Experiment 2

In experiment 2, 280 7 d turkey poults were randomly allocated to 56 battery cages (as described in experiment 1) in a completely randomized design. There were a total of five poults per cage (replicate), seven replicates per treatment; and cage served as an experimental unit. Birds were assigned to one of eight dietary treatments (described in experiment 2). Performance (BW, FI, FCR, AME and NR) and gut measurements were the same as in experiment 1.

5.4.5 Chemical analysis

The excreta samples collected in experiments 1 and 2 were oven dried for 72 hours at 55°C for dry matter determination. After drying, samples from each replicate were pooled together for analysis. Chemical analyses were as described in chapter 3 (section 3.3.3).

5.3.6 Calculations

The formulas by Scott and Hall (1998) were used for calculating AME and NR. Refer to chapter 3 (section 3.3.4) for details on calculations.

5.3.7 Statistical analysis

All experiments were analyzed using Proc GLM (General Linear Model) of SAS version 9.2 (SAS Institute Inc, 1996). A cage of five poults was considered an experimental unit. In experiment 1, the data were analyzed as a 3 x 2 factorial arrangement. There were three levels of wDDGS (0, 15 and 30%) and two enzymes [none (E-), enzyme cocktail (E+)]. In experiment 2, data were analyzed as a 2 x 2 x 2 factorial arrangement. There were two wDDGS levels (15 and 30%), two enzymes (E- and E+) and two processing methods (EX- and EX+). Means were considered statistically significant when $P \leq 0.05$. Duncan's Multiple Range Test was used for separation of mean values when differences were significant.

5.4 Results

The wDDGS used in the diet formulation contained 89.2% DM, 36.0% CP, 4.57% fat and 6.29% crude fiber. Analyzed nutrient compositions of the respective dietary treatments (all experiments) are shown in Table 5.2. The overall health of turkey poults was excellent and no poults were removed from the two studies. Overall, all treatments (experiment 1 and 2) average 21 d BW was 675±41.08 g, FI was 47.68±3.58 g/b/d and FCR was 1.32±0.05. There was no interaction between main effects for intestinal measurements relative to BW; hence only main effects are reported. To remove confounding effects of particles size, the wDDGS (EX-/EX+)

were ground before feed mixing. Mean particle size (Dgw±Sgw; data not shown) was higher for raw wDDGS (1330±48.9 micron; unground); whereas the ground unextruded and extruded wDDGS (485± 41.96 microns; EX- , 415±68.8 microns; EX+) used in the diets were not different.

Table 5.2. Analyzed nutrient composition of dietary treatments fed to determine the effects extrusion and wet feeding of wheat DDGS (wDDGS) with and/or without an enzyme cocktail (0.5 g/kg) on turkey hen poults (as fed)

Item	Dry Matter (%)	AME (kcal/kg)	Protein (%)
Experiment 1			
0% E-	90.69	2786	29.57
0% _E+	90.83	3124	29.54
15% _E-	91.21	3086	29.13
15% _E+	91.08	3211	29.63
30% _E-	91.32	3260	30.04
30% _E+	91.37	3329	29.35
Experiment 2			
15%_EX-_E-	91.21	3086	29.13
15%_EX-_E+	91.08	3211	29.63
15%_EX+_E-	91.46	3280	29.50
15%_EX+_E+	91.37	3144	29.45
30% _EX-_E-	91.32	3260	30.04
30% _EX-_E+	91.37	3329	29.35
30% _EX+_E-	91.71	3317	29.77
30% _EX+_E+	91.52	3335	29.49

AME = apparent metabolizable energy
wDDGS =wheat distillers dried grains with solubles
E+=Enzyme cocktail (Superzyme;1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase, 7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase)
E-= No enzyme addition
EX+= extruded diet
EX-= non-extruded diet

5.4.1 Experiment 1: Effects of wheat distillers dried grains with solubles with and/or without an enzyme cocktail on performance and nutrient availability

With the exception of the FCR (7-21 d), average BW (21 d) and FI (7-21 d; g/b/d) were higher for 0% wDDGS (Table 5.3). The inclusion levels of 15% and 30% wDDGS were not different from each other. The results indicate no effects of E; neither were there interactions between level of wDDGS inclusion and E on poult performance.

Table 5.3. Experiment 1. Effects of wheat distillers dried grains with solubles with and/or without an enzymes cocktail (0.5 g/kg) on the body weight (BW), feed intake (FI) and feed conversion ratio (FCR) of turkey hen poults (7-21 d)

Item	df n	Body weight 21 d (g/b)	Feed intake (g/b/d)	FCR (g/g)
Levels of wDDGS	1	*	**	P=0.16
0% wDDGS	14	703[a]	50.1[a]	1.31
15% wDDGS	14	681[b]	47.6[b]	1.30
30% wDDGS	14	664[b]	46.7[b]	1.32
Enzymes	1	NS	NS	NS
None (E-)	21	688	48.6	1.31
Enzyme (E+)	21	677	47.6	1.31
Level of wDDGS *Enzyme	1	NS	NS	NS
SEM		12.7	1.01	0.010

SEM=Standard error of means
Means with different superscripts within the same column are significantly different *$P \leq 0.05$; **$P \leq 0.01$
wDDGS=Wheat distillers dried grains with solubles
Enzyme cocktail (Superzyme;1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase, 7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase)

There were no interactions between inclusion level of wDDGS and enzyme for relative measurements of the intestinal tract segments. Proventriculus weight, gizzard weight, duodenal length and ileal length were significantly higher for 30% wDDGS (Table 5.4). There was also a tendency (P=0.06) for jejunum length to be longer for 30% wDDGS. There was no effect of enzyme on gut segment size, with a numerical (P=0.09) increase in relative ileal length with the enzyme cocktail supplementation.

Table 5.4. Experiment 1 Effects of wheat distillers dried grains with solubles with and/or without an enzyme cocktail (0.5 g/kg) on intestinal measurement relative to 21 d body weight.

Item	Proventriculus weight (%)	Gizzard weight (%)	Duodenal length (%)	Duodenal length (%)	Jejunum length (%)	Jejunum weight (%)	Ileal length (%)	Ileal weight (%)	Caeca length (%)	Caeca weight (%)
Levels of wDDGS	*	*	**	NS	P=0.06	NS	*	NS	NS	NS
0% wDDGS	0.445[a]	2.26[b]	2.86[b]	0.919	6.84[b]	1.62	6.89[b]	1.18	4.30	0.746
15% wDDGS	0.454[b]	2.24[b]	3.09[a]	0.886	7.27[ab]	1.54	7.11[ab]	1.16	4.35	0.856
30% wDDGS	0.476[a]	2.40[a]	3.13[a]	0.913	7.36[a]	1.58	7.44[a]	1.16	4.40	0.694
Enzymes	NS	NS	NS	NS	NS	NS	P=0.09	NS	NS	NS
None (E-)	0.465	2.33	2.97	0.903	7.06	1.59	6.99	1.17	4.39	0.750
Enzyme (E+)	0.452	2.27	3.09	0.909	7.26	1.57	7.30	1.16	4.31	0.781
SEM	0.0063	0.062	0.087	0.0537	0.132	0.063	0.221	0.043	0.154	0.132

SEM=Standard error of means
Means with different superscripts within the same column are significantly different *$P \leq 0.05$; **$P \leq 0.01$
wDDGS=Wheat distillers dried grains with solubles
Enzyme cocktail (Superzyme;1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase, 7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase)

Nitrogen retention and diet AME at 21 d are presented in Table 5.5. At 21 d, the NR was lower for 0% wDDGS as compared to either 15 or 30%, which were not different from each other. The NR was improved by enzyme supplementation. There was an interaction (Figure 5.1) between wDDGS inclusion level and enzyme supplementation for NR determined at 21 d. The interaction indicates that enzymes increased NR of 0% wDDGS diets, but had no effect when 15 or 30% wDDGS were included. The AME determined at 21 d increased with each increase in wDDGS inclusion and there was an overall improvement with enzyme supplementation.

Table 5.5. Experiment 1. Effects of wheat distillers dried grains with solubles with and/or without an enzyme cocktail (0.5 g/kg) on 21 d nitrogen retention and apparent metabolizable energy of turkey hen poults

Item	Nitrogen retention (%)	AME (kcal/kg)
Levels of wDDGS	**	**
0% wDDGS	52.6[b]	2955[c]
15% wDDGS	57.3[a]	3149[b]
30% wDDGS	58.3[a]	3295[a]
Enzymes	**	**
None (E-)	53.9	3045
Enzyme (E+)	58.2	3221
Level of wDDGS *Enzyme	**	**
SEM	0.70	28.8

SEM=Standard error of means
Means with different superscripts within the same row are significantly different *$P \leq 0.05$; **$P \leq 0.01$
wDDGS=Wheat distillers dried grains with solubles
Enzyme cocktail (Superzyme;1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase, 7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase)

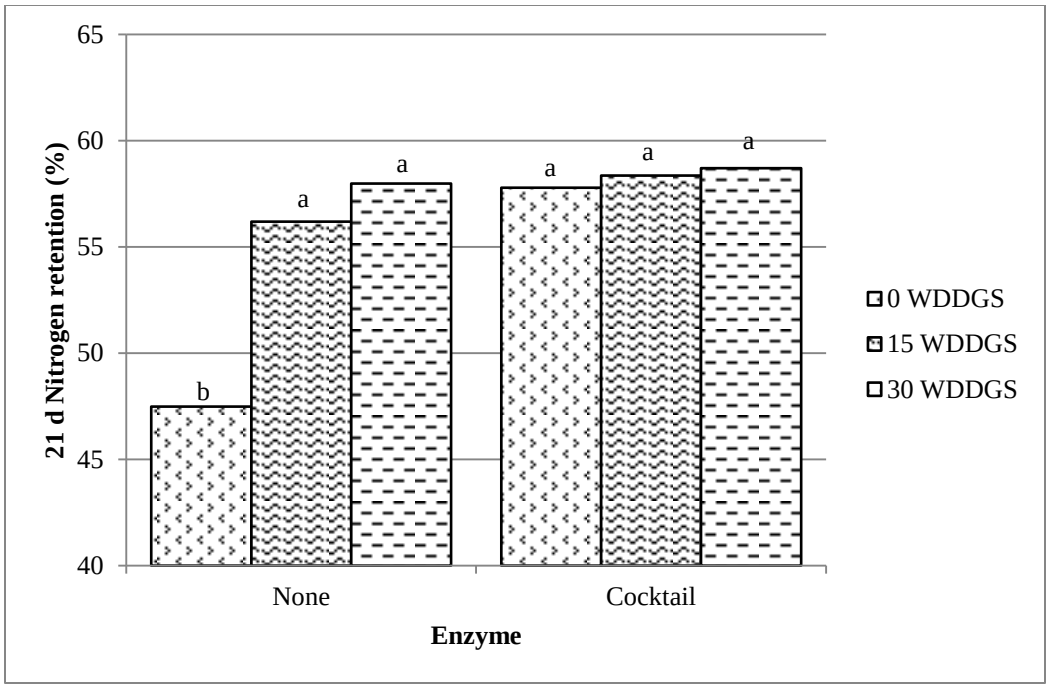

Figure 5.1. Experiment 1. The interaction between wheat distillers dried grains with solubles (wDDGS) levels (0, 15and 30%) and enzyme (none, cocktail) on nitrogen retention (%).
Bars without common letters (a, b) are significantly different (P<0.05)

There was an interaction (Figure 5.2) for AME at 21 d between wDDGS inclusion and enzyme supplementation. The interaction indicate that enzyme supplementation significantly improved the AME of diets with 0 or 15% wDDGS, but did not improve the AME in the 30% wDDGS diet.

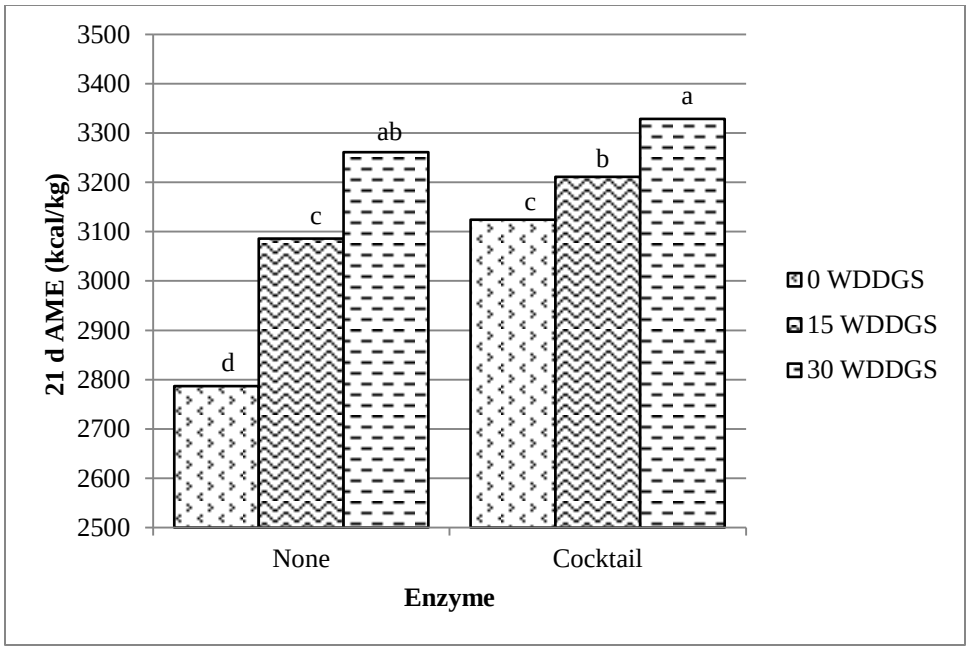

Figure 5.2. Experiment 1. The interaction between wheat distillers dried grains with solubles (wDDGS) levels (0, 15and 30%) and enzyme (none, cocktail) on apparent metabolizable energy.
Bars without common letters (a-d) are significantly different (P<0.05)

5.4.2 Experiment 2: Effects of wheat distillers dried grains with solubles (with or without extrusion of wDDGS) and with and/or without an enzyme cocktail on performance and nutrient availability

The average performance (7-21 d) of turkey poults is shown in Table 5.6. There were no 2-way or 3-way significant interactions reported for performance variables. There was a significant effect of inclusion level on 21 d BW and 7-21 d FCR; both were negatively affected by 30% as compared to 15% wDDGS inclusion. There was no effect of inclusion level on FI. There were no effects of enzyme or extrusion of wDDGS used in the diets on BW, FI or FCR.

Table 5.6. Experiment 2. Effects of wheat distillers dried grains with solubles with and/or without an enzyme cocktail (0.5 g/kg) with and/or without extrusion process on mean body weight (BW), feed intake (FI) and feed conversion ratio (FCR) of turkey hen poults (7-21 d)

Item	df n	Body weight 21d (g/b)	Feed intake (g/b/d)	FCR (g/g)
Levels of DDGS	1	*	NS	*
15% wDDGS	28	681[a]	47.7	1.30[a]
30% wDDGS	28	657[b]	46.5	1.33[b]
Enzymes	1	NS	NS	NS
None (E-)	28	674	47.7	1.32
Enzyme (E+)	28	664	46.5	1.32
Processing	1	NS	NS	NS
Non-extruded (EX-)	28	673	47.2	1.31
Extruded (EX+)	28	665	47.0	1.32
Level of wDDGS *Enzyme	1	NS	NS	NS
Level of wDDGS *Processing	1	NS	NS	NS
Enzyme*Processing	1	NS	NS	NS
Level*Enzyme*Processing	1	NS	NS	NS
SEM		15.4	1.39	0.020

*, NS Indicates Significance at P<0.05 and not significant respectively

Means with different superscripts within the same column of same factor are significantly different *$P \leq 0.05$

SEM=Standard error of means.

Enzyme cocktail (Superzyme;1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase, 7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase)

There were no interactions between inclusion level (15 or 30%) of wDDGS, extrusion or enzyme supplementation for relative gut segment size estimates (Table 5.7). The only effect of wDDGS inclusion level was an increase in relative proventriculus weight and ileal length with increased wDDGS. Enzyme supplementation decreased relative proventriculus and gizzard weight and had a numerical effect of increasing relative duodenal (P=0.07) and caecal (P=0.09) length. The extrusion of wDDGS in the diets reduced both relative proventriculus and gizzard weights, but had no effect on the measurements for the other gut segments.

Table. 5.7. Experiment 2. Effects of wheat distillers dried grains with solubles with and/or without an enzyme cocktail (0.5 g/kg) with and/or without extrusion on intestinal measurement relative to 21d body weight. There were no significant 2-way or 3-way interactions.

Item	Proventriculus weight (%)	Gizzard weight (%)	Duodenal length (%)	Duodenal weight (%)	Jejunum length (%)	Jejunum weight (%)	Ileal length (%)	Ileal weight (%)	Caeca length (%)	Caeca weight (%)
Levels of wDDGS	*	NS	NS	NS	NS	NS	*	NS	NS	NS
15% wDDGS	0.449[b]	2.23	3.16	0.911	7.24	1.52	7.09b	1.15	4.45	0.797
30% wDDGS	0.464[a]	2.30	3.18	0.923	7.38	1.54	7.48a	1.18	4.51	0.699
Enzymes	*	*	P=0.07	NS	NS	NS	NS	NS	P=0.09	NS
None (E-)	0.465[a]	2.32[a]	3.11	0.900	7.32	1.56	7.27	1.16	4.37	0.776
Enzyme (E+)	0.449[b]	2.21[b]	3.24	0.935	7.30	1.50	7.30	1.17	4.58	0.721
Processing	*	*	NS	NS	NS	NS	NS	NS	NS	NS
Non-extruded (EX-)	0.465[a]	2.31[a]	3.17	0.945	7.34	1.52	7.25	1.17	4.49	0.737
Extruded (EX+)	0.449[b]	2.22[b]	3.17	0.889	7.27	1.54	7.33	1.16	4.48	0.758
Pooled SEM	0.0102	0.060	0.097	0.0585	0.207	0.063	0.215	0.045	0.172	0.1156

SEM=Standard error of means

Means with different superscripts within the same column are significantly different *$P \leq 0.05$; **$P \leq 0.01$

wDDGS=Wheat distillers dried grains with solubles

E+=Enzyme cocktail (Superzyme;1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase,7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase

At 21 d there was no effect of inclusion level on NR (Table 5.8) but there was a positive response to 30% as compared to 15% wDDGS inclusion on AME (21 d). The results indicated no effect of enzyme. There was a numerical (P=0.10) improvement in NR for diets with extruded wDDGS and this was significant for AME.

Table 5.8. Experiment 2. Effects of wheat distillers dried grains with solubles with and/or without an enzyme cocktail (0.5 g/kg) with and/or without extrusion on 21 d diet nitrogen retention and apparent metabolizable energy of turkey hen poults

Item	Nitrogen retention (%)	AME (kcal/kg)
Levels of wDDGS	NS	**
15% wDDGS	58.2	3181
30% wDDGS	58.8	3310
Enzymes	NS	NS
None (E-)	58.4	3235
Enzyme (E+)	58.7	3258
Processing	P=0.10	*
Non-extruded (EX-)	57.8	3222
Extruded (EX+)	59.2	3269
Level of wDDGS *Enzyme	NS	NS
Level of wDDGS *Processing	NS	NS
Enzyme*Processing	*	**
Level*Enzyme*Processing	*	**
Pooled SEM	0.40	13.9

SEM=Standard error of means
Means with different superscripts within the same column are significantly different *$P \leq 0.05$; **$P \leq 0.01$
wDDGS=Wheat distillers dried grains with solubles
E+=Enzyme cocktail (Superzyme;1100 units/g of xylanase, 375 units/g of glucanase, 350 units/g of invertase, 700 units/g of protease, 1650 units/g of cellulase, 7250 units/g of amylase, 230 unit/g of mannanase and 25 units/g of galactanase)

There were 2-way (enzyme x extrusion) and 3-way (inclusion level of wDDGS x enzyme x extrusion) interactions for 21 d NR and AME (Figures 5.3 and 5.4). For NR, the 3-way interaction signifies that for 15% wDDGS inclusion there was no effect of enzyme when wDDGS were not extruded and a negative effect when wDDGS was extruded. At 30% inclusion there were no effects of enzyme or extrusion on NR.

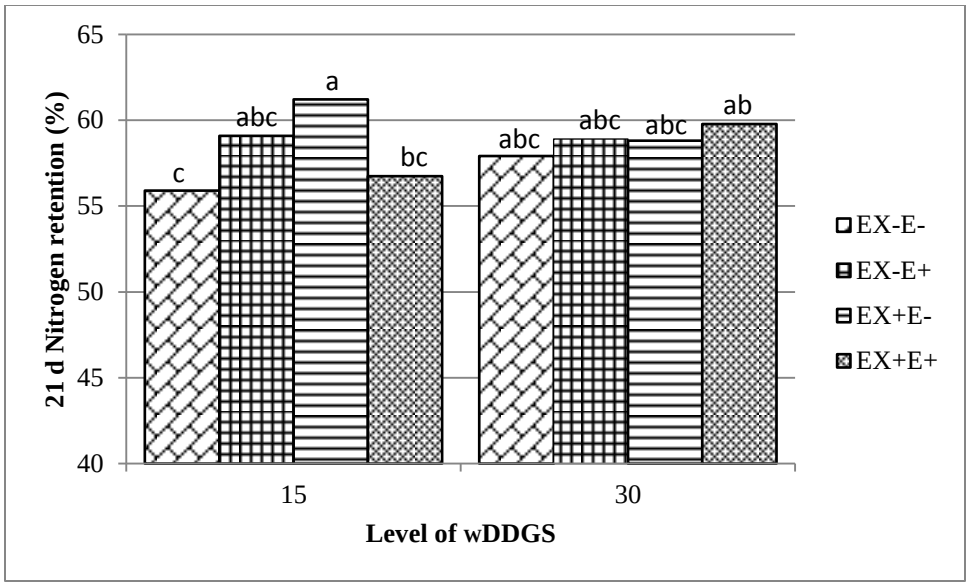

Figure 5.3. Experiment 2. The interaction between wheat distillers dried grains with solubles (wDDGS) levels (15and 30%), enzyme (none, cocktail) and extrusion (EX-, EX+) on nitrogen retention (%). Bars without common letters (a, b) are significantly different (P<0.05)
EX-E-=No extrusion / no enzyme
EX-E+=No extrusion / enzyme
EX+E-=Extrusion / no enzyme
EX+E+=Extrusion / enzyme

The 3-way interaction for AME (Figure 5.4) indicate that at 15% inclusion there was a positive effect of enzyme with no extrusion, whereas there was a negative effect of enzyme with extrusion. For the 30% wDDGS inclusion there were no effects of diet regardless of whether the wDDGS were extruded or not.

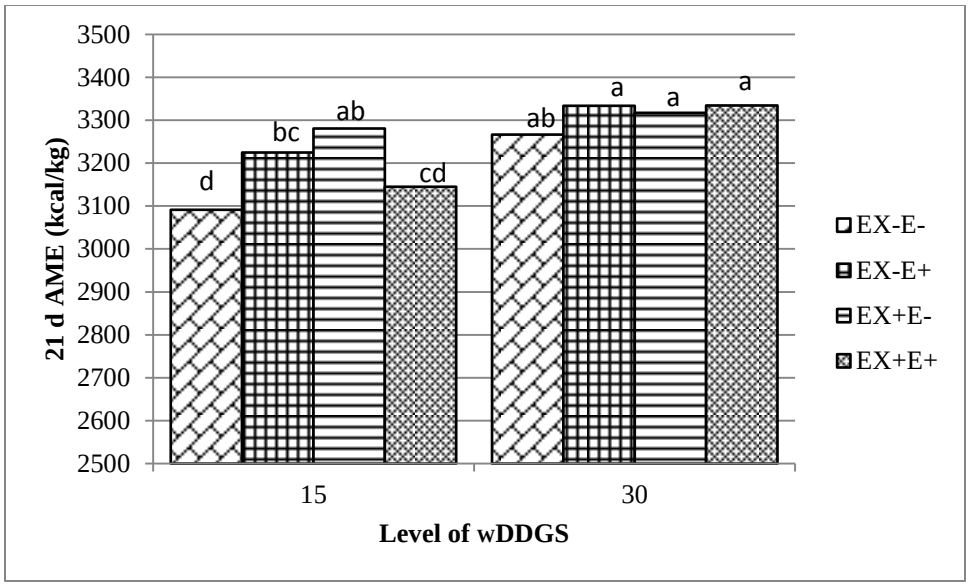

Figure 5.4. Experiment 2. The interaction between wheat distillers dried grains with solubles (wDDGS) levels (15 and 30%), enzyme (none, cocktail) and extrusion (EX-, EX+) on apparent metabolizable energy. Bars without common letters (a-c) are significantly different ($P<0.05$)
EX-E-=No extrusion / no enzyme
EX-E+=No extrusion / enzyme
EX+E-=Extrusion / no enzyme
EX+E+=Extrusion / enzyme

5.5 Discussion

Generally, due to depression in performance associated with feeding diets high in fiber; feeding higher levels of wDDGS to poultry is of concern (Lee et al., 2003). The present study evaluated the potential for processing and/or enzyme supplementation to improve nutrient intake and utilization of diets containing up to 30% wDDGS. The results indicated significantly lower performance by feeding up to 30% wDDGS but birds remained healthy throughout the experiment. This is consistent to the findings of Abdel-Raheem et al. (2011), Roberson (2003) and Wang et al. (2007). Monogastrics lack the enzymes necessary to breakdown the complex cell wall structure of fiber; hence depression in performance was not surprising. Knudsen (2001) has reported that higher intake of dietary fiber decreases the total tract digestibility, which then results in an increased percentage of the energy being digested in the large intestines. This consequently resulted in less monosaccharide absorption in the small intestines and more short

chain fatty acids being produced in the large intestines (Knudsen, 2001; Svihus et al., 2012). Additionally, increasing heat treatments during processing may result in increasing levels of soluble NSP's that are associated with higher digesta viscosity and consequently poor nutrient utilization.

Alternatively, during ethanol production, most of the starch in cereals is converted to ethanol (Chevanan et al., 2008; Kerr and Shurshon, 2013). The extraction of fat is also commonly practiced in modern ethanol plants using corn as a substrate (Wisner et al., 2013); however, this is not practiced when wheat is used as a substrate. These practices can significantly depress the energy content of the co-product. The lower performance with diets containing 30% wDDGS in the present study contradicts our earlier findings (Opoku et al., 2012). However, the wDDGS used in both studies were sourced from the same processing facility. It is critical to understand that, processes involved in the production of DDGS are variable within and between processing plants. There is evidence of variability in nutrient level and/or digestibility in wDDGS obtained from the same plant in Western Canada (Lumpkins et al., 2004; Oryschak et al., 2010b). With the inconsistencies in DDGS, the assumed nutritional composition is often inaccurate (Liu, 2012), thus leading to errors in feed formulations.

The use of carbohydrase enzyme or a multi-enzyme complex is intended to release sugars from fibrous carbohydrates for higher absorption and assimilation in the small intestines (Meng et al., 2002; Emiola et al., 2009 and Ziemer et al., 2012). Nonetheless a multi-enzyme supplement was not effective on performance when feeding higher levels of wDDGS. This effect was also observed in our previous study (Opoku et al., 2012); although this study compared individual supplementation of either protease and/or β-mannanase. Omogbenigun et al. (2004) on the other hand, showed improved performance when an enzyme cocktail (cellulase,

galactanase, mannanase, and pectinase) was supplemented in wheat based diets fed to pigs. Omogbenigun et al. (2004) attributed this to an improvement in ileal fiber digestibility that improves overall nutrient absorption and minimizes lower tract fermentation and loss of nutrients.

Nutrient digestibility (21 d) were in some instances higher for 30% wDDGS. This is similar to that reported by Oryschack et al. (2010a) and Opoku et al. (2012). The higher fiber content in the diet containing 30% wDDGS might have resulted in slower transit time of digesta and better interaction of endogenous enzymes with feed in the gut and thereby improved digestibility (Lee et al., 2003). However, the lack of a direct link between improvements in digestibility and performance are confusing, but have been reported previously (Opoku et al., 2012) and may be associated with overall limitations in nutrient intake or higher costs of digesting low quality ingredients.

There is a possibility of greater gut fermentation in diets with 30% wDDGS because of the higher fiber. Lee et al. (2003) noted that size of the gut is influenced positively by increasing dietary fiber. Hence the fermentation capacity of the caeca (Svihus et al., 2013) might be responsible for the increased digestibility; as an increased length was reported for both experiment 1 and 2. It is also likely that a proportion of the total energy and amino acid requirements consumed in the diet were directed to maintaining the increase gut size. In experiment 1, a 7.38% and 4.34% longer Ileal length for 30% wDDGS compared to 0 and 15% wDDGS, respectively was found. A reduced ceca length, 2.19% (0% wDDGS) and a 1.18% (15% wDDGS) was also reported. Similar to experiment 1, ileal (5.24%) and caeca (1.50%) segments were longer for bird's fed 30% as compared to 15% wDDGS in experiment 2.

Beneficial interactions among carbohydrases (Choct et al., 2004; Tahir et al., 2008) have been reported. Similarly, supplementation of a multi-enzyme complex containing a combination of xylanase, amylase, protease or β-glucanase, xylanase and amylase accounted for an increase nutrient digestibility in broilers and pigs (Inborr et al., 1993; Olukosi et al., 2010). The interaction in inclusion level and enzyme for 21d NR levels in experiment 1 could be related to differences in the diet ingredient matrix. This may explain why enzymes were more effective at lower as compared to higher inclusion levels of wDDGS. The increased AME for 0 and 15% due to interaction with enzyme supplemented suggests that cocktail enzyme is probably more efficient; but this could be dependent on percentage of less digestible ingredient included.

Enzyme supplementation according to Choct et al. (1996) directs most of the fiber degradation in the gut towards caeca instead of the lower digestive tract. With its fermentation capability, the caeca is a source of volatile fatty acid (VFA) production in birds (Svihus et al., 2013). The numerically (P=0.09; 4.59%) higher relative caeca length (experiment 2) could be due to enzyme supplementation compared to diets fed without enzymes; might signify an increase in fiber fermentation. Svihus et al. (2013) indicated that the VFA production would result in an increase in energy digestibility. However, no improvement in energy digestibility was found with enzyme supplementation in experiment 2. We did not measure VFA production in the present study.

Currently, there is inadequate information on feeding extruded wDDGS to poultry (Oryschack et al., 2010a; Oryschack et al., 2010b). To our knowledge this experiment is the first to report the impact of extrusion on wDDGS fed to turkey hens. Extrusion did not positively affect the performance of turkey poults in the present study. Amornthewaphat et al. (2005) reported a significant improvement in performance when broilers were fed extruded corn.

Garcia et al. (2003) showed that steam cooking at 99±2°C of barley base diet to chicks improved performance until 8 d of age, but the effect disappeared thereafter. Additionally, Vukic-Vranjes et al. (1994) observed a negative effect on average daily gain and feed conversion ratio in 21 d broilers fed wheat and maize extruded diets. The reason for the inconsistencies among researchers is unidentified but might be related to the ingredients, the conditions (time, temperature and/or moisture) applied for extrusion and the age of the birds (Gonzalez-Alvarado et al. 2007; Gracia et al., 2009). Application of heat solubilizes the fibrous component of ingredient (Garcias et al., 2008; de Vries et al.., 2012), which increased the solubility of dietary fiber resulting in increased intestinal viscosity (Mateos et al., 2002 ; Gracia et al., 2003; Scott et al., 2003). Apparently, this may impair effective nutrient utilization in turkey hen poults. Unfortunately, we do not have data on intestinal viscosity in the present study.

Hydrothermal treatments modify the physicochemical structure of the diet, including the fiber component (Bjorck and Asp, 1983), destroy microbes in feed ingredients and facilitate the accessibility of enzymes thereby improving their digestibility (Said, 1996; Oryschak et al., 2010b; de Vries et al., 2012). The results in experiment 2 (Table 5.8) indicate a higher 21 d digestibility (both NR and AME) when diets contained extruded wDDGS rather than unextruded wDDGS. This observation agrees with data from Gracia et al. (2003) and Gonzalez-Alvarado et al. (2007). Similarly Oryschack et al. (2010a, b) has reported an increase in apparent ileal digestibility of amino acids by single screw (triticale DDGS) and twin screw extrusion (wheat and corn DDGS), in the respective studies. Contrarily, Vukic-Vranjes and Wenk (1995) reported that energy utilization was negatively affected when broilers were offered diet containing extruded barley. The improvement in 21 d digestibility may signify beneficial effects with

extruded wDDGS in the diets. It is surprising as to why the improvement in nutrient digestibility with extruded diets was not mirrored in improved performance.

Gracia et al. (2003) reported an increase in nutrient digestibility when steam cooked barley based diet was supplemented with an enzyme complex containing xylanase, protease and amylase. Similarly, Vukic-Vranjes and Wenk (1995) has indicated a significant positive effect in an enzyme supplemented extruded barley base diet on AMEn of broilers. The present study recorded no significant impact of enzyme supplementation on extruded wDDGS. The use of hemicellulase enzymes (Smith et al., 2006; Bruce et al., 2007) with a combined effect of processing during ethanol production might have resulted in hydrolyzing NSPs. It is also reported by Chervanan et al. (2010) that individual feed ingredients demonstrate distinct behavior during processing. Hence, detailed information on the composition of the fiber-fraction and digestibility of its components would assist in identifying and understand the modifications that occur during processing (de Vries et al., 2012). This would ensure an appropriate selection of source and level of specific cell wall degrading enzymes to better match specific substrates (Zijlstra et al., 2010).

In summary, the current studies show that wDDGS is a potential energy and protein source for turkeys. However, with the high fiber content of this feedstuff, more studies must be conducted to gain a better understanding of how the high fiber content might influence the feed value of wDDGS for young turkeys. It would seem futile to continue the application of enzymes to wDDGS if detailed analysis is not done to determine the major fiber fractions; and subsequently the appropriate cocktail enzyme combination and levels. These experiments have illustrated some beneficial effects of processing (extrusion) and/or an enzyme cocktail supplementation on nutrient digestibility; but no improvement in performance were observed.

Although outside the bounds of this study, it would be interesting if future research investigates the potential of altering processing variables for extruding wDDGS.

6.0 WET FEEDING AND EXTRUSION OF WHEAT DISTILLERS DRIED GRAIN WITH SOLUBLES FED TO TURKEY HEN POULTS

6.1 Abstract

This study evaluated if wet feeding (WF; 1.2 mL water: 1.0 g feed) and/or extrusion (EX) would overcome the limitations associated with wheat distillers dried grains with solubles (wDDGS) nutrient intake and improve young turkey hen performance (7-21d). Significant improvements ($P<0.05$) in BW, FI and FCR were observed with WF. A tendency ($P=0.08$) for significant interaction on 21 d AME was recorded. There was a negative effect of extrusion on AME determined at 21d. Voluntary feed consumption is improved when diets are fed as wet. Extrusion of wDDGS was not beneficial for performance.

6.2 Introduction

Ethanol is produced by fermentation and distillation processes using the sugar and starch from cereal grains. Wheat distillers dried grains with solubles (wDDGS) is a co-product obtained when using wheat as the major raw material. The poultry industry is utilizing more wDDGS as a source of protein and to a limited extent, energy. However, high fiber in wDDGS is associated with reduced intake and digestion of nutrients. Wet feeding and extrusion are not commonly practiced in poultry, but are used by other animal industries to better utilize ingredients.

With the shorter passage rate of digesta in the intestines of poultry; it is important for feed to be digested quickly for better nutrient absorption and utilization (Forbes, 2003). Wetting of diets increases the solubility and easy penetration of digestive juice; rendering the feed more accessible to digestive enzymes (Yasar and Forbes, 2001; Forbes, 2003). It also appears to reduce the size and importance of the gizzard as feeding wet diets significantly reduced gizzard size (Afsharmanesh et al, 2006). Scott believes that voluntary FI is influenced by the rate of

water hydration of the feed and that ingredients can vary significantly in rate of hydration and therefore impact rate of passage and subsequent FI (Scott, 2002; Scott and Silversides, 2003). The efficiency of wet diets in promoting higher growth in broilers has been demonstrated in wheat-based diets (Scott, 2002; Scott and Silversides, 2003). However it is uncertain whether such improvements can be achieved for young turkeys fed diets high in wDDGS.

Extrusion involves the use of high temperature, pressure and moisture to change the physicochemical profile of ingredients (Fallahi et al., 2013). Reduction in antinutritional factors, enhanced palatability and digestibility are some beneficial effects of extrusion (Ayadi et al., 2011). Significant improvement in amino acids digestibility were reported when broilers were fed diets containing extruded triticale DDGS (Oryschak et al., 2010a). Currently, not much is known about feeding extruded diets to poultry; and it is unknown whether extrusion of the wDDGS to turkeys is useful (Opoku et al., 2013).

We hypothesize that wet feeding and/or extrusion can improve the feed value of wDDGS for poultry. This research will therefore look at determining if wet feeding and/or extrusion will positively impact the utilization of wDDGS diets by turkey hen poults.

6.3 Materials and methods

All procedures involving animal handling and testing were reviewed and approved by the University of Saskatchewan Committee on Animal Care and Supply (animal use protocol no. 19940248) and followed the principles established by the Canadian Council on Animal Care (1993).

The wDDGS used in the current experiment was supplied by Husky (Lloydminster, Saskatchewan, Canada). A basal diet containing 30% wDDGS (non-extruded; EX- and extruded; EX+; chapter 5; Table 5.1) was formulated to either meet or exceed the nutrient requirements of Hybrid Converter turkey starter diet (http://www.hybridturkeys.com/hybrid-

resources/nutritional-guidelines). For details on dietary composition and extrusion process refer to chapter 5 (Table 5.1).

A total of 102 one-day old Hybrid Converter turkey hens (Lilydale Hatchery, Edmonton, Alberta) were placed in battery cages at the University of Saskatchewan Poultry Centre. Poults were kept in groups of 10 for the first 7 d. They had free access to a standard wheat soybean turkey starter diet (Similar to chapter 5; section 5.3.4). On d 7, individually weighed turkey poults (wing banded) were assigned to 22 battery cages measuring 29.2 cm (height) × 48.3 cm (depth) × 83.8 cm (width; providing 1010 cm^2/bird at 21 d) in a completely randomized design. Poults were then assigned to 4 different dietary treatments [30% wDDGS (EX-; dry) 30% wDDGS (EX-; wet), 30% wDDGS (EX+; dry) 30% wDDGS (EX+; wet)]. A total of seven cages of five poults were fed the respective (EX-, EX+) dry diets and four cages of four poults also fed wet diets. A standard brooding temperature starting from 32°C from 0 d and gradually reduced to 23°C at 21d was used. The birds were exposed to 18L:6D (L;light; D;dark), with a light intensity of 10-20 lux.

The 30% (EX-, EX+) wDDGS based diets were pre-tested with various levels of added water to provide the same porridge-like consistency as recommended by Yasar and Forbes (1995); with no water layer forming over the feed that may lower voluntary feed intake. A ratio of 1.2 litre water per 1.0 kg diet was found to be optimum for the diets tested. The wet diets were prepared daily and allowed to equilibrate for ~10 minutes before feeding, in feeders lined with disposable plastic bags. The remaining wet feed in each feeder was weighed and discarded the next day. Moisture lost to evaporation during the 24 h access to the wet feed was not accounted for. In the respective wet diets, the amount consumed was monitored and expressed on a dry as-fed basis by subtracting the added water from the wet feed consumed.

On d 7, 14 and 21; BW and FI were recorded. Feed conversion ratio corrected for mortality was calculated. At the end of the study (21d) two birds were humanely killed by cervical dislocation for each replicate. For intestinal measurements, similar procedure described in chapter 3 (section 3.3.2.1) was used. For apparent metabolizable energy (AME) and nitrogen retention (NR) determination; see chapter 3 (section 3.3.3) for details on excreta collection and chemical analysis. Calculation of AME and NR were based on those used by Scott and Hall (1998); as described in chapter 3 (3.3.4).

The experiment was analyzed using Proc GLM (General Linear Model) of SAS version 9.2 (SAS Institute Inc, 1996)). A cage of either five or four birds (in the case of wet diets) was considered an experimental unit. The data were analyzed as a 2 x 2 factorial arrangement, with two processing (EX-, EX+) and two feed forms (dry and wet). Means were considered statistically significant when $P \leq 0.05$.

6.4 Results

The chemical composition of the wDDGS used in the diet formulation was 36.0% CP, 89.2% DM, 4.57% fat and 6.29% crude fiber. Analyzed nutrient compositions of the non-extruded diet are 91.3% DM, 3260 kcal of AME/kg and 30.0 % CP; extruded diets contained 91.7% DM, 3317 kcal of AME/kg; 29.8% CP. To remove confounding effects of particle size, all wDDGS (EX-/EX+) were ground before feed mixing. Mean particle size (Dgw$\pm$Sgw; data not shown) was higher for raw wDDGS (1330±48.9 micron; unground) compared to the ground; whereas the ground material used in diet preparation (485±41.96 microns; EX- , 415±68.8 microns; EX+) were not different.

The overall health of the turkey poults was excellent as no mortality was recorded. The average body weight of birds at 7 d was not different between treatments. Growth performance during the experimental period averaged 695±55.5 g/b (BW), 50.9±5.64 g/b/d (FI) and of

1.35±0.04 (FCR). The average performance on 21 d BW, FI and FCR were improved for main effects of wet diets compared to dry diets (Table 6.1). There was no effect on processing main effects, and neither was there interaction between main effects on poults performance. There were no interactions between main effects on intestinal measurements relative to body weight (Table 6.2). Higher proventriculus and gizzard weights were found for EX-. All gut measurements (except duodenal weight, ceca weight and jejunum weight) were higher for dry diets.

Table 6.1. Effects of extrusion and wet feeding of diets with 30% wheat distillers dried grains with solubles on body weight, feed intake, FCR of turkey hen poults (7-21 d)

Item	Body weight 21 d (g/b)	Feed intake (g/b/d)	FCR (g/g)
Processing	NS	NS	NS
Non-extruded	695	51.0	1.31
Extruded	696	50.9	1.34
Feed form	**	**	**
Dry	661[b]	47.4[b]	1.34[a]
Wet	755[a]	57.2[a]	1.29[b]
Processing* Feed form	NS	NS	NS
Pooled SEM	11.8	1.20	0.009

SEM=Standard error of means
Means with different superscripts within the same row are significantly different *$P \leq 0.05$; **$P \leq 0.01$
wDDGS=Wheat distillers dried grains with solubles

Table 6.2 Effects of extrusion and wet feeding of wheat distillers dried grains with solubles on intestinal measurement relative to 21 d body weight

Item	Proventriclus weight (%)	Gizzard weight (%)	Duodenal length (%)	Duodenal weight (%)	Jejunum length (%)	Jejunum weight (%)	Ileal length (%)	Ileal weight (%)	Caeca length (%)	Caeca weight (%)
Processing	*	**	NS	NS	NS	NS	NS	NS	NS	NS
Non-extruded	0.469[a]	2.37[a]	2.97	0.900	6.89	1.60	7.08	1.11	4.25	0.721
Extruded	0.444[b]	2.16[b]	3.08	0.954	6.97	1.55	7.22	1.14	4.44	0.703
Feed form	*	**	**	NS	**	*	*	**	**	P=0.09
Dry	0.468[a]	2.35[a]	3.18[a]	0.913	7.35[a]	1.51[b]	7.36[a]	1.19[a]	4.60[a]	0.742
Wet	0.435[b]	2.13[b]	2.75[b]	0.951	6.20[b]	1.69[a]	6.78[b]	1.01[b]	3.92[b]	0.660
Pooled SEM	0.0108	0.057	0.093	0.0507	0.201	0.047	0.187	0.047	0.139	0.041

SEM=Standard error of means

Means with different superscripts within the same row are significantly different *$P \leq 0.05$; **$P \leq 0.01$

wDDGS=Wheat distillers dried grains with solubles

Processing and feed form did not affect NR at 21 d; neither was there an interaction between feed form (wet/dry) and processing (Table 6.3). The AME was higher for EX+ as compared to EX- diets. There was the tendency (P = 0.08) for interaction between feed form and extrusion on AME (21 d) to be significant (Figure 6.1). This signifies that there was a reduced AME when non-extruded diets were wetted. Whereas pre-wetting of diets improved AME of extruded diets.

Table 6.3. Effects of extrusion and wet feeding diets with 30% wheat distillers dried grains with solubles on 21 d nitrogen retention and apparent metabolizable energy of turkey hen poults

Item	Nitrogen retention (%)	AME (kcal/kg)
Processing	NS	**
Non-extruded	56.7	3238
Extruded	58.6	3329
Feed form	NS	NS
Dry	58.4	3289
Wet	56.2	3275
Processing* Feed form	NS	P = 0.08
Pooled SEM	0.71	16.0

SEM=Standard error of means
Means with different superscripts within the same row are significantly different *$P \leq 0.05$; **$P \leq 0.01$
wDDGS=Wheat distillers dried grains with solubles

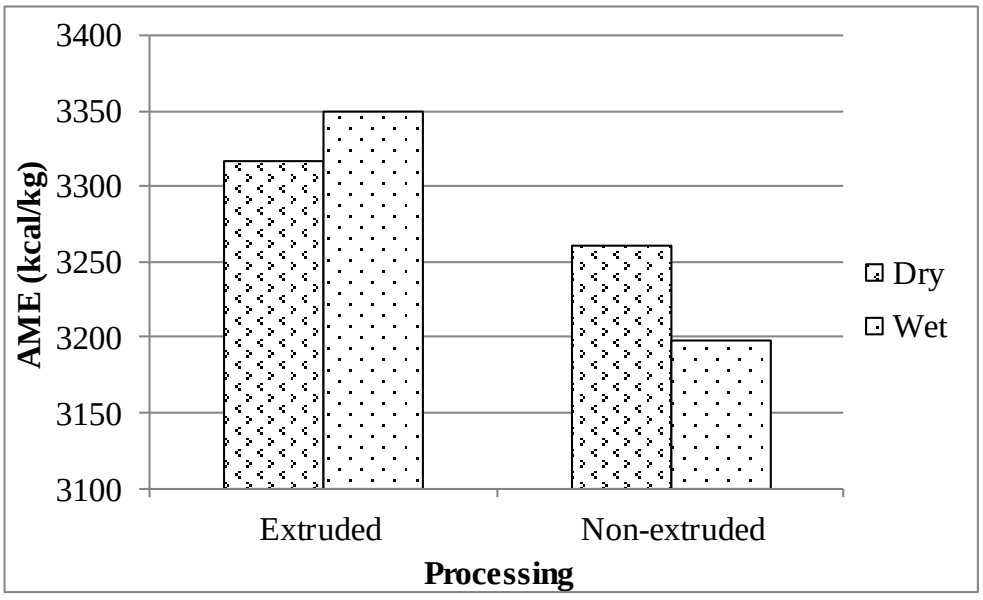

Figure 6.1: The interaction between processing (EX-, EX+) and feed (dry, wet) on apparent metabolizable energy (P=0.08)

6.5 Discussion

Nutritionists are seeking alternative means of reducing the limitations associated with feeding higher levels of co-products (e.g. DDGS) to poultry. If this restriction is related to slower diet hydration because of the physicochemical profile of wDDGS, then overall feed intake may be improved with pre-wetting of diets as explained by Scott (2002) and Scott and Silversides (2003). The present results in terms of feed intake and body weight with wet diets are consistent with findings of Yalda and Forbes (1995, 1999), Scott (2002) and Scott and Silversides (2003). Comparing the present study with the requirements of the hybrid converter turkey standards (http://www.hybridturkeys.com/en/hybridproducts/mainstreamproducts/converter/~/media/Files/Hybrid/Converter/CS_Converter_Females_WEB_FINAL.ashx); wet diets recorded similar 21 d BW (0.76 kg; hybrid standard vs 0.76 kg; wet diets) and FCR (1.27; hybrid standard vs 1.29; wet diets).

The improved performance of turkeys fed wet wDDGS diets clearly indicated the limitation associated with feeding dry wDDGS diets as suggested by Scott and Silversides (2003) when dry wheat-based diet was fed to broilers. It could probably be related to the faster diffusion of digestive juice to speed up digestion and hence higher feed intake (Yasar and Forbes, 2001; Forbes, 2003). Addition of water to feed reduced the time required for hydration in the gut and increased rate of digestion and passage of digesta through the digestive tract (Forbes, 2003). Scott (2002) had hypothesized that, "water hydration rate and/or capacity is important in determining feed intake". Forbes (2003) was with the opinion that the increase in FI of birds offered a choice of wet feed compared to dry once is due to the high palatability (i.e., in a situation where skim milk is used in wetting the mash) of wet feeds.

The higher digestibility of dry diets with lower performance might be attributed to the higher maintenance requirements of the significantly higher gut. This signifies that nutrients were directed towards maintenance of the gut. Even though there was no effect of pre-wetting on increasing AME and NR, the improved performance might be an indication of a higher and/or improved nutrient utilization with wet wDDGS diets. The efficient nutrient utilization with feeding wet diets might be attributed to activation of endogenous enzymes in the feed. Forbes (2003) suggested that increased nutritional value might not necessarily be linked to addition of the water per se, but transformation such as fermentation of the diet (i. e., before feeding) may have occurred through the addition of water. According to Ziemer et al. (2012) the fermentation of fiber by microorganisms enhances the availability and absorption of short chain fatty acids.

Scott (2002) observed that wet feeding of some wheat-based diets was excessively high and subsequent feed conversion lowered. It was believed that in some instances wet feeding made intake and digesta passage rate too easy and the birds did not have to digest the diet as efficiently to get the total nutrients required to support higher growth. With wet feeding the bird's intake increased and if it is possible for the bird to eat enough feed it will do so and may not to fully digest the diet. This activity could also explain the significantly reduced gut size (relative to 21 d body weight) for wet fed diets, except duodenal weight (4.0% reduction; not significant) compared to dry diet. A negative effect on nutrient utilization was also shown in this experiment with extruded diets. Gracia et al. (2003) noted that steam cooking at 99±2°C of barley diet enhanced chicks performance up to 8 d of age, but decreased afterwards. Vukic-Vranjes et al. (1994) also observed a negative effect on performance of 21 d broilers fed wheat and maize extruded base diets. Additionally, Vukic-Vranjes and Wenk (1995) reported negative energy utilization when broilers were offered diet containing extruded barley. This according to

Mateos et al. (2002) might be attributed to the increase in intestinal viscosity and subsequently poor performance. Factors such as ingredient (physical and chemical characteristics), the processing conditions (time, temperature and/or moisture) and birds' age might be responsible for these results (Gonzalez-Alvarado et al. 2007; Garcia et al., 2008). The interaction between feed form and processing (21d AME; P=0.08) however suggests a positive effect of wetting on extruded wDDGS. The reason for this is not known and further investigation into this is required.

In conclusion, the advantages of wet feeding using wDDGS diets have been illustrated in this study. Wet feeding allows birds to consume and digest more diet. Further research with wet wDDGS diets is required to clearly understand the non-improvement in nutrient digestibility with increase voluntary feed intake and improved performance.

7.0 OVERALL DISCUSSION AND FUTURE DIRECTIONS

Generally, feeding higher levels of wDDGS to poultry has not been encouraged due to concerns about inconsistencies in its nutritional value and high fiber content. Notwithstanding these limitations, the poultry industry and nutritionist utilizes these co-products as substitutes for cereals; in response to the rate at which these grains are being converted to ethanol. The objective was to determine the nutritional value of an alternative feed ingredient from the bioethanol industry for turkeys; and contribute to the limited information available on using higher levels of wDDGS diets fed to turkey hens. It was of interest to evaluate if subjecting wDDGS to enzyme application and processing technology would increase its feed value, safety, competitiveness and sustainability in the poultry industry.

Beneficial effects of feeding DDGS have been reported in the literature (Wang et al., 2007; Olukosi et al., 2010). The current research (excluding chapter 5) has indicated that feeding high levels (30% wDDGS) of wDDGS was not detrimental to the overall performance of turkeys when fed. Hence this supports the hypothesis that turkeys have the capacity to utilize the nutrients in wDDGS; and equally perform on diets with up to 30% wDDGS (chapter 3, 4). Some depression in performance was however noticed in some instances (chapter 5) by feeding higher level (30%) of wDDGS. It is quite interesting that nutrient digestibility was improved at this higher level. The reason(s) for this is not known; hence further research is required to understand this. It could however, be speculated that unknown limitations may be present; as this negative effect was not seen when diets (30% wDDGS) were fed as wet (chapter 6). We also postulate that assumed nutrient composition for co-products are often inaccurate and could be a potential challenge in feed formulation.

Using enzymes in poultry nutrition is a widely discussed topic between nutritionist and producers; particularly, with the increased use of co-products (e. g., DDGS) in least cost feed

formulations. Independent use of enzymes (protease and β-mannanase) was not effective in the current research. Likewise, the cocktail enzyme consisting of xylanase, glucanase, invertase, protease, cellulase, amylase, mannanase and galactanase failed in improving turkey performance. The study by Ghazi et al. (2003) has indicated that the beneficial effect of protease could partially be related to the contribution from an α-galactosidase which was added to the diets.

The percentage of mannan in the wDDGS used was not known; as no analysis was done to determine mannan level. However, research has indicated that the yeast used in fermentation of DDGS contains approximately ~6% mannan content that could be of potential threat in the DDGS (Tucker et al., 2004; Radfar et al., 2013). Enzyme efficacy, according to Zijlstra et al. (2010), is proportionally related to substrate availability. On the other hand, Choct (2006) had earlier on stated that a suitable match of an enzyme activity to its substrate is not an assurance of enzymes efficacy. According to Choct (2006) "substrate specificity depends largely on the source of the enzyme". The unexpected depression in single enzyme supplementation may on the other hand indicate that further degradation of the protein or β-mannanase was not beneficial or required. All enzymes were fed as a single dose. Designing the research to test for different enzyme concentrations with varying levels of wDDGS would have been a better approach. However, the research facility does not currently have the capacity to accommodate such kinds of trials (i. e., limited space).

We hypothesized a positive effect on extruding wDDGS. Extrusion of wDDGS was not beneficial. Oryschack et al. (2010a, b) used extruded triticale and extruded wheat or corn in their respective studies. These authors showed improvement in amino acid digestibility by feeding these diets to broilers. Extruded diets in Oryschack's experiments were fed in the starter phase, similar to what was done in the current experiment. The results indicated no positive influence

of feeding extruded DDGS in the starter phase, notwithstanding the increase in nutrient digestibility. This was consistent with what was seen in the current research (chapter 5). We are unaware of any scientific publication on feeding extruded DDGS to turkey. The contradiction between nutrient digestibility and performance is confusing and makes the data difficult to explain. It could be inferred that other factor (s) were responsible for this. Detailed research into this is therefore required.

The marked increases in performance (as indicated in chapter 6) with wet feeding are similar to those observed by others and indicate that voluntary feed consumption is improved when diets are pre-wetted before feeding. The explanation for this is still not clear, but suggests that pre-soaking diets increases speed of digestion and enables birds to consume more and meet their nutrients requirements for rapid growth. There is every reason to believe that wet feeding has great potential, but practical evidence on larger scale is still scarce. Limitations to the adoption of wet feeding include the requirement for different feeding systems and concerns about feed spoilage. Forbes (2003) noted that, wet diets are more susceptible to mold growth, which is undesirable and transmits pathogens such as Salmonella. More research is required, if possible on commercial bases/standards. Subject to initial cost of installation and mold growth, wet feeding is an appropriate practice that can be adopted in the poultry industry, if limitations are addressed.

In order to select an appropriate enzyme to target specific substrates in wDDGS, future research should be directed towards estimating the level and nature of fibre in wDDGS. To understand the interaction between processing (i.e., extrusion) and wDDGS requires further research in altering processing variables for extruding wDDGS

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9.0 APPENDICES

Appendix 1

Poultry Science (2012) 91:16 (Abstract)

Poultry Science Conference, July 9-12, 2012, Athens, Georgia, USA

The effect of wheat distillers dried grains with solubles fed with or without protease or β-mannanase on the performance of turkey hen poults

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Pressure to reduce the use of fossil fuels has resulted in an increased dependency on the use of grains for ethanol production. Distillers dried grains with solubles (DDGS), a co-product from ethanol production, can be used in poultry diets, but little information is available on the use of wheat DDGS diets for turkeys. An experiment was conducted to investigate the effect of wheat DDGS on growth performance and gut measurements of turkey hen poults in a 2×3 factorial arrangement. Two basal diets formulated to meet or exceed nutrient requirements for Hybrid Converter turkey starter diets contained either 0 or 30% DDGS. The DDGS used in the diet formulation contained 35.9% protein and 4.57% fat. Each basal diet was divided into three portions and supplemented with no enzyme, protease (0.125 g/kg) or β-mannanase (0.5 g/kg). A total of 144, 7 day old Hybrid Converter female turkey poults were randomly distributed to provide 4 birds for each of 6 replicate cages per treatment. There was no mortality in the study. There were no effects of treatments or interactions on feed intake from 7 to 21d. However, 30% DDGS inclusion ($P<0.05$) improved 21d body weight and feed conversion ratio. The relative (to 21d body weight) empty proventriculus and gizzard weight, duodenal, jejunum, ileal and caecal length were not different due to DDGS and/or enzyme. There were significant main effects and interactions on AME determined for the diets, overall the highest energy determined was for

30% DDGS with no enzyme (i.e., enzymes did not significantly improve 30% DDGS diets) and lowest for 0% DDGS with no enzyme (enzymes significantly improved AME of 0% DDGS diets). In conclusion, wheat DDGS can be incorporated in the turkey starter diet as high as 30% without detrimental effects on performance. The data failed to demonstrate a benefit of exogenous protease and β-mannanase enzyme on turkey performance regardless of the inclusion of DDGS.

Key words: wheat, distillers dried grains with solubles, turkey, protease, β-mannanase.

Appendix 2

Poultry Science (2012) 91:137 (Abstract)

Poultry Science Conference, July 9-12, 2012, Athens, Georgia, USA

The effect of dietary glycerol in the starter phase on turkey hen production

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Glycerol, a co-product from bio-diesel production, has the potential to be an energy source for turkey production. Presently, it is not recognized as a feed ingredient by the Canadian Food Inspection Agency; and therefore this research was undertaken to build a scientific basis for the acceptability of glycerol in turkey feeding. In the present study, a total of 96 Hybrid Converter female turkey poults were fed a wheat and soybean meal basal diet containing different levels of glycerol (0, 2.5, 5.0 and 7.5 %) from 7 to 21 d of age. Diets in mash form were balanced with different levels of canola oil, to maintain a similar energy content for all diets. The four diets were each randomly assigned to six cages (4 poults per cage). Feed and water were available on an ad libitum basis. There were no adverse effects of glycerol inclusion on growth performance. There was no mortality in the study. Data was analyzed by linear regression. There was no significant difference in 21 d body weight (g) or feed intake (g/b/d). However, there was a significant linear effect of glycerol level on 7-21d feed conversion ratio (y=1.54-0.0114*glycerol level; R^2=0.31; P<0.05). There were no significant effects of glycerol level on AME (kcal/kg diet). There was a (P=0.06) linear effect of glycerol on nitrogen retention (y=53.9+0.88*glycerol level; R^2=0.15). Relative to 21d body weight gut measurements (i.e. empty gizzard and proventriculus weight, and duodenal, jejunum, ileal and caeca length) were not affected (P>0.05) by the level of glycerol. In conclusion, the data suggest that glycerol can be incorporated in the diet of hen turkeys as high as 7.5% without detrimental effect on growth performance.

Key words: glycerol, turkey poults, growth performance, feed conversion efficiency, gut measurement.

Appendix 3

Poultry Science (2013) 92:37 (Abstract)

Poultry Science Conference, July 22-25, 2013, San Diego, California, USA

Evaluation of inclusion level of wheat distillers dried grains with solubles with and/or without protease and β-mannanase on performance and water intake of turkey hens.

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It is becoming increasingly common to use higher levels of wheat distillers dried grains with solubles (wDDGS) in poultry diets. The objective was to determine the effect of level of inclusion of wDDGS with or without enzyme supplementation on performance and rate of water intake on turkey hens (0-72d). Two diets (0 or 30% wDDGS) were formulated to meet the nutrient requirements of the Hybrid Converter turkey. Diets were then mixed to obtain different levels of inclusion of 0, 15 or 30% wDDGS. The 30% diet was divided into 3 fractions and 2 fractions supplemented with either protease (P+; 0.126g/kg) or β-mannanase (M+; 0.05g/kg). All diets were fed ad libitum as mash. All 700 0d turkey hens were randomly allocated into groups of 35 birds per replicate with 4 replicates floor pens per treatment, in a completely randomized design. Water intake per pen was recorded beginning at 7d. There was no effect of dietary treatment on feed intake. Body weight of turkey hens (28-52d grower) was significantly higher for 30%P+ as compared to 0 or 15% diets; but was not different than 30% or 30%M+ diets. Feed:gain($P<0.01$; 28-52d), and total feed:gain ratio ($P<0.05$; 0-72d) was significantly improved for birds fed 30% regardless of enzyme treatment compared to 0% and 15% levels. Water intake tended to be higher ($P=0.08$) between 7-28d for 30%P+ diets. Similarly, water intake of birds fed 30%P+ was higher ($P<0.05$; 28-52d) and total water intake ($P=0.06$; 7-72d) tended to be higher than other treatments. Similarly water:gain ratio (52-72d) was higher ($P<0.05$) for 30%P+. To our knowledge, this experiment is the first to report the impact of wDDGS on water intake. As

high as 30% wDDGS can be substituted in turkey hen diets. No impact of protease or β-mannanase was found on performance of turkey hens fed 30% wDDGS.

Key words: Wheat, distillers dried grains with solubles, water intake, turkey, enzymes

Appendix 4

Poultry Science (2013) 92:117 (Abstract)

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The effects of extrusion of wheat distillers dried grains with solubles with and/or without enzymes on performance turkey hen poults.

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High fiber potentially reduces the utilization of wheat distillers dried grain with solubles (wDDGS) for poultry and therefore it was of interest to determine if processing or dietary enzymes could improve nutritional value. A total of 10 dietary treatments, 0% wDDGS [non-extruded (EX-) with and/or without enzyme (E+/ E-)], 15% wDDGS [(EX-/EX+), (E+/E-)] and 30% wDDGS [(EX-/EX+), (E+/E-)] were fed to assess their impact in the starter phase. In the respective treatments, only the wDDGS was extruded using a twin-screw extrusion. Temperature at the end of the barrel was 118°C, with retention of 1 min 55 sec, total moisture was 25% and 33 Bar pressure. Diets met or exceeded the nutrient requirements of the Hybrid Converter turkey. The respective diets were supplemented with an enzyme cocktail (0.5g/kg) containing xylanase, glucanase, invertase, protease, cellulose, amylase, mannanase and galactanase. Diets were fed ad libitum to a total of 350 7d old hen poults from 7-21d; each treatment was assigned 7 cages of 5 poults in a completely randomized design. Data was collected on poult performance and 21d intestinal tract measurements expressed on relative body weight basis. There was no significant effect of diet on feed intake and feed:gain ratio. Average weight gain (21d) were significantly higher ($P<0.05$) for 0% (E-) as compared to 30% [(EX-/EX+), (E+/E-)] wDDGS treatments; but not different than the 0% level with enzyme) or 15% wDDGS treatments regardless of extruder or enzyme application. Significant ($P<0.05$) effect of

diet was observed for proventriculus weight, gizzard weight, duodenal length and ileal length. Intestinal segments were higher for the 30% compared to the 0%. Incorporation of wDDGS in turkey hen diets is found to be an appropriate practice. Neither extrusion nor enzyme was effective in improving the nutritional value of wDDGS as indicated by growth performance.

Key words: Wheat distillers dried grains with solubles, extrusion, enzyme, turkey, growth performance

Appendix 5

34th Western Nutrition Conference (2013) Page 193 (Abstract)

Western Nutrition Conference, September 24-26, 2013, Saskatoon, Saskatchewan, Canada

Wet feeding and extrusion of wheat distillers dried grain with solubles fed to turkey hen poults

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The poultry industry is utilizing more wheat distiller's dried grains with solubles (wDDGS) as a valuable source of protein and, to a limited extent, energy. However, high fiber in wDDGS is associated with reduced intake and digestion of nutrients. Wet feeding (WF) and extrusion (EX) are not commonly practiced in poultry, but is used by other animal industries to better utilize ingredients. Hence, a study was conducted to evaluate if EX and/or WF of diets with 30% wDDGS would overcome the limitations associated with wDDGS nutrient intake and enhance poults performance. The study compared unextruded (EX-) and extruded (EX+; twin screw extruder; final barrel temperature 118C; 1 min 55 sec retention; 25% moisture; and 33 Bar pressure) wDDGS added at 30% to a common basal diet; the final diets were then fed with or without 1.2 litre water per 1.0 kg diet to produce a porridge-like consistency prepared fresh daily. Diets containing 30% wDDGS either met or exceeded the nutrient requirements of the Hybrid Converter turkey and were fed ad libitum to a total of 102 7d old hen poults from 7-21d; 7 cages of 5 poults were fed the respective (EX-, EX+) dry diets and 4 cages of 4 poults also fed wet diets in a completely randomized design. There was no significant main effect of EX of wDDGS on poults 21 d body weight, feed intake (DM basis) or FCR. Significant improvements ($P<0.05$) in body weight, feed intake and FCR were observed with WF. There was significant interaction between WF and EX on 14d NR and AME. There was the tendency ($P=0.08$) for interaction to

be significant on 21 d AME. The main effect of EX was significantly higher ($P<0.05$) for EX-on AME (21d). The significant increase in performance with WF indicates that voluntary feed consumption is improved when diets are pre-wetted before feeding. The WF allows birds to consume and digest more diet and warrants further research to develop practical means of feeding poultry. Extrusion of wDDGS was not beneficial.

Key words: Wheat distillers dried grains with solubles, extrusion, wet feeding, turkeys